CROOKS
LAB

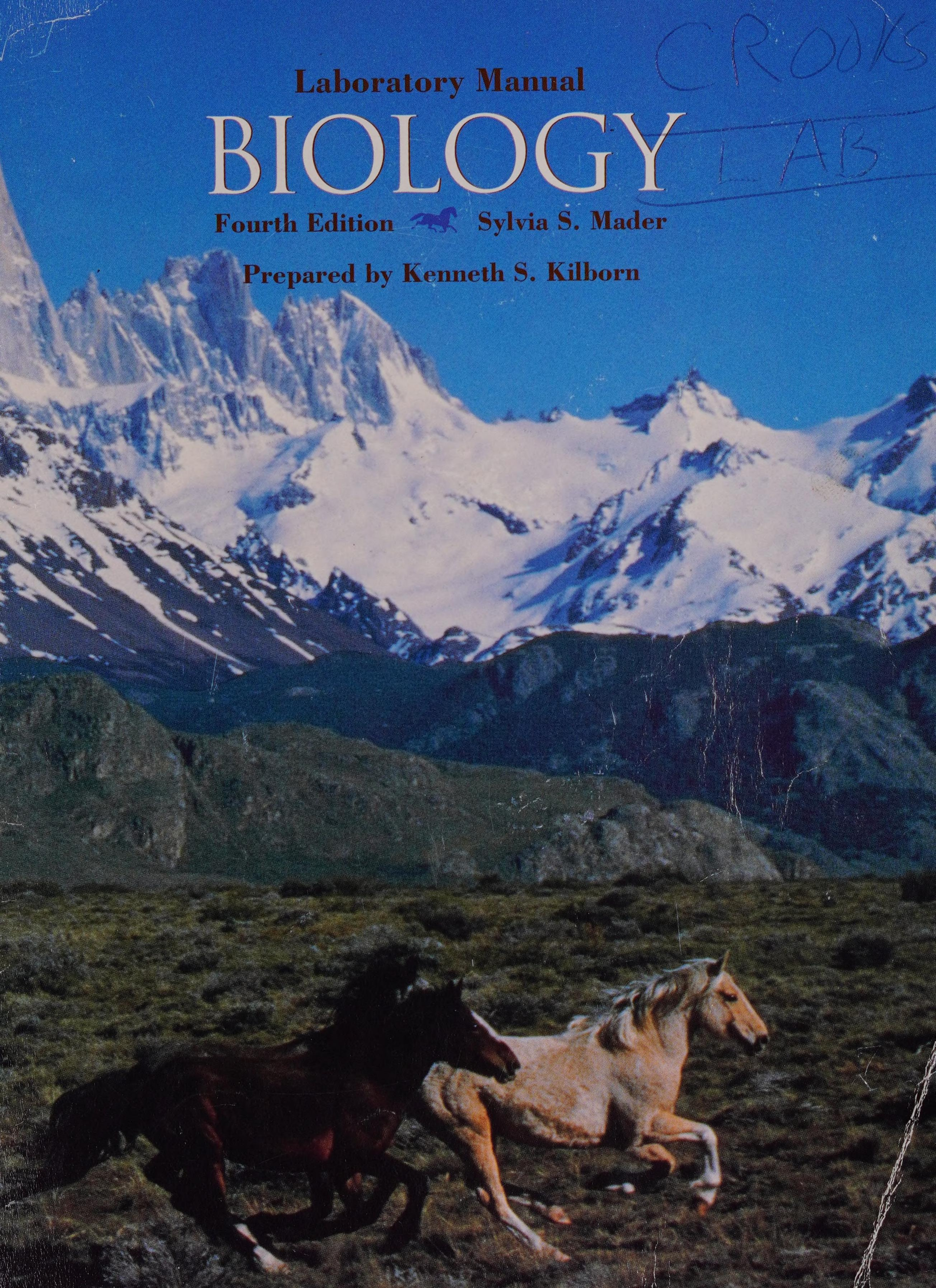
Laboratory Manual

BIOLOGY

Fourth Edition

Sylvia S. Mader

Prepared by Kenneth S. Kilborn



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Laboratory Manual

BIOLOGY

Fourth Edition

Sylvia S. Mader

Revised by

Kenneth S. Kilborn
Shasta College

WCB Wm. C. Brown Publishers
Dubuque, Iowa • Melbourne, Australia • Oxford, England

Some of the laboratory experiments included in this text may be hazardous if materials are handled improperly or if procedures are conducted incorrectly. Safety precautions are necessary when you are working with chemicals, glass test tubes, hot water baths, sharp instruments, and the like, or for any procedures that generally require caution. Your school may have set regulations regarding safety procedures that your instructor will explain to you. Should you have any problems with materials or procedures, please ask your instructor for help.

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*I **Incubation**—Required; student should *start* these sections at the beginning of the laboratory
L **Living Material**—Advanced order and timely delivery
F **Fresh Material**—Local purchase close to time of use
C_{2d} **Culture or Growth**—Required; hours (h), days (d), weeks (w)
D **Demonstration**—Instructor conducted

31 Animal Behavior	44	371	2.0–3.0+	L,F,C_{1-3wk}
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Preface

This *Laboratory Manual* accompanies *Biology,* by Sylvia S. Mader, which covers the entire field of general biology from an evolutionary perspective. This manual, like the text, introduces major concepts and applies them to specific areas of biology. Each laboratory is referenced to the appropriate chapter in *Biology,* but the laboratories can easily be coordinated with other biology texts. All exercises have been tested for student interest, preparation time, estimated time of completion, and feasibility.

The author wishes to acknowledge the review of the *Laboratory Manual* and the *Laboratory Resource Guide* by Diane Morris, Biology Laboratory Preparations Supervisor, and graduate assistants Keith Hagen, Mari Beth Johnston, Heidi Kass, and Jeffry Rain, under the guidance of Professor Patrick Trihey of Bemidji State University.

New to this Edition

The contents now includes an estimated time to complete and special requirements to aid in scheduling, ordering, and preparation.

"Microscopy and Linear Measurement" (Laboratory 1) has an augmented explanation of metric conversions and a new section describing the use of the binocular dissecting microscope.

In "Chemical Composition of Cells" (Laboratory 2), additional test tubes provide immediate testing and observation to allow comparison with the incubated test tubes. More guidance has been given to help students arrive at conclusions.

"Mendelian Genetics" (Laboratory 7) has expanded definitions and a new formatting for clarification.

In "Human Genetics" (Laboratory 8), the karyotype chromosome drawings are now sticky backed to facilitate handling. New definitions have been added, plus the pedigree problems and the table of human traits are reformatted. Solutions to the pedigree problems are in the "Exercises—Expected Results" portion of the *Laboratory Resource Guide.*

"Biotechnology—Gene Cloning" (Laboratory 10) is reformatted.

"Biotechnology—Gel Electrophoresis" (Laboratory 11) is entirely new and includes DNA and protein electrophoresis.

"Diversity I: Bacteria, Protista, and Fungi" (Laboratory 14) contains additional sections exploring the diversity of red algae, brown algae, and pond water organisms.

"Diversity IV: Primitive Invertebrates," "Diversity V: Protostomes," and "Diversity VI: Deuterostomes" (Laboratories 17, 18, and 19) now have clarifying definitions along with charts for comparison of diversity within each phylum. *Note:* If these laboratories are used as separates, selection of all 3 would aid continuity.

"Basic Mammalian Anatomy," "Comparative Vertebrate Anatomy," and "The Nervous System" (Laboratories 23, 24, and 27) have improved illustrations. *Note:* If laboratory separates are used, selection of all 3 laboratories should be chosen because each has some material on the fetal pig dissection.

"Symbiotic Relationships" (Laboratory 32) has new diagrams for clarification.

"Analysis of an Ecosystem" (Laboratory 33) has been completely reformatted to allow for recording and analysis of abiotic and biotic factors in both terrestrial and/or aquatic ecosystems. This activity can be adapted to field or laboratory use.

Formatting Improvements

Icons highlight each procedure that requires active student participation as well as being placed at the top of the lab. The following icons are used to designate extra time needed for a lab, a student activity, a demonstration, and if incubation is needed: ⓣ ⓢ ⓓ ⓘ.

All tables are reformatted to allow more space for student explanations.

There are numerous new full-color illustrations and photographs.

Laboratory review questions have been reformatted to allow for easier grading. The majority of these questions test for understanding rather than rote memory. Answers appear in the *Laboratory Resource Guide*. Some instructors may choose to grade these questions as a measure of the student's understanding, and other instructors may choose to use these questions as a student self-test. If you use the Laboratory Review as a self-test you can copy the "Answers—Laboratory Review" section of the *Laboratory Resource Guide* and distribute them to students who have purchased the *Laboratory Manual* or the separates of the *Laboratory Manual*.

In this edition, the list of materials have been expanded and placed in the appendix. These same lists also appear in the Resource Guide for the manual.

The *Laboratory Resource Guide* has been greatly expanded. See its preface and contents for details.

To the Instructor

Several features in this *Laboratory Manual* allow adaptation to a variety of course orientations. There are a sufficient number of exercises to permit a choice of activities over the length of the course. The estimated time for completion and special requirements are now given in the contents and in the *Laboratory Resource Guide* to aid in advanced planning. Many exercises have optional sections if time permits or have sections (such as phylum diversity and pond water study) that can be expanded to meet curriculum needs. Many sections of this manual may be performed as demonstrations rather than as student activities, thereby shortening the time required to cover a particular concept. Students work with living material during some part of almost all laboratory exercises. The majority of exercises are designed to be self-contained and easily performed by a number of laboratory sections in succession.

Appendixes

Appendix A—Preparing a Laboratory Report/Laboratory Report Form

If you require your students to write a laboratory report, this appendix provides students a format to follow. The lab report is divided into Introduction, Procedure, Results, and Discussion. There are instructions and examples for each section, and a report form is included. You may wish to begin this course by asking students to complete the report form for one particular exercise within a given laboratory and end the course by requiring a completely prepared laboratory report for the entire exercise, using the instructions and form simply as a guide. If students are particularly advanced, you may wish to add more sections to the outline, particularly later on. For example, a more lengthy discussion could also include alternative interpretations, identification of assumptions, and countering of possible criticism.

Appendix B—Metric System

For each common metric unit the name, value, and symbol are given.

Appendix C—Classification of Organisms

The revised classification of organisms used in *Biology* and the *Laboratory Manual* is included.

Practical Examination Answer Sheets

Forms to be used for practical examinations are provided.

Laboratory Resource Guide

The expanded *Laboratory Resource Guide* is essential for instructors and laboratory assistants and is available free, to adopters of the *Laboratory Manual,* in bound form or as separates.

The new features of the *Laboratory Resource Guide* include:

An expanded list of suppliers—inside front cover

A separate section for "Laboratory Preparation and Instructions," which for each laboratory includes:

—estimated time, which varies considerably with the amount of optional and additional material;

—complete materials lists, with sections for hardware, chemicals, microscope slides (prepared), preserved material, living material, fresh material, models, and texts;

—catalog numbers and approximate quantities for needed materials;

—specific instructions for instructors and laboratory assistants, including hints for preparing and organizing materials.

A separate section for the "Laboratory Exercises and Expected Results," including a repeat of the question or data table with the expected results in *italics*.

A separate section for "Laboratory Review Answers."

Instructor and Laboratory Assistant Feedback

If you have any suggestions for how this *Laboratory Manual* could be improved, you can send your comments to:

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To the Student

Special care has been taken in preparing the *Laboratory Manual* for *Biology* so that you will *enjoy* the laboratory experience as you *learn* from it. The instructions and discussion are written clearly so you can understand the material while working through it. Student aids are designed to help you focus on important aspects of each exercise.

Student Learning Aids

Student learning aids are carefully integrated throughout this manual. Each laboratory begins with a list of *learning objectives* that set out the goals of each laboratory session and help you review the material for a laboratory practical or any other kind of exam. Then comes an *introduction,* which establishes the rationale for the coming work and reviews much of the necessary background information required for comprehension of upcoming experiments. *Icons* bring attention to sections that require active participation. Throughout, *space* is provided for recording answers to questions and the results of investigations and experiments.

Each laboratory ends with a set of review questions covering the day's work. Appendices at the end of the book provide useful information on preparing a laboratory report, the metric system, and classification of organisms. Practical examination answer sheets are also provided.

Laboratory Preparation

Read each exercise before coming to the laboratory. *Study* the introductory material and the experimental procedures. If necessary, to obtain a better understanding *read* the corresponding chapter in your text. If your text is *Biology,* by Sylvia S. Mader, see the text *chapter reference* in the contents at the beginning of this *Laboratory Manual* or the corresponding chapter in any other biology text.

Explanations and Conclusions

Learning to formulate explanations or conclusions is an important objective of this *Laboratory Manual.* It requires a synthesis of information from a variety of sources, including the following:

Results of experiments or tests, yours and other groups in the class—if your data is different from other groups in your class, do not erase your answer, add the other answer in parentheses.

The knowledge of the underlying principles behind the test or experiment—obtain this information from the introduction to the chapter or section of the laboratory and the corresponding section of your text.

The understanding of the nature of the response to the specific ingredient you have added or to the test you have conducted. *Note:* ingredients can be contaminated or procedures incorrectly followed, resulting in reactions that seem inappropriate. If this occurs, consult with other students and your instructor to see if you should repeat the experiment.

Be sure that you are truly writing an explanation or conclusion, not just a restatement of the observations.

Legend of Icons

Ⓢ **Student Activity**—Perform this specific task.

Ⓘ or Ⓣ **Incubation** or **Time**—Allow the designated amount of time for this activity. Start these activities at the *beginning* of the laboratory, proceed to other activities, and return to these when the designated time is up.

Ⓓ **Demonstration**—The instructor will conduct an actual demonstration experiment, or will have this activity as a display.

Student Feedback

If you have any suggestions for how this *Laboratory Manual* could be improved, you can send your comments to:

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Laboratory

1

Microscopy and Linear Metric Measurement

Learning Objectives

Students should be able to

1. name the linear units of measurement in the metric system, state the value in meters of each linear unit, and convert between various units;
2. describe 3 differences between the compound light microscope and the electron microscope;
3. name and give the function of the basic parts of the compound light microscope;
4. describe how the slide of the letter *e* provides information on the inversion of the image in the compound light microscope;
5. explain how the slide of colored threads provides information on the depth of field;
6. calculate the diameter of the field and the total magnification for both the low and high power lens systems;
7. list, in proper order, the steps used for bringing an object into focus with the compound light microscope;
8. name the 3 kinds of cells studied in this exercise;
9. state 2 differences between onion epidermal cells and human epidermal cells.

Introduction

Comprehending the relative size of objects and the structures within them aids in understanding the complicated organization within biological systems. The metric system is the universal system used by biologists. This exercise considers only the linear measurements[1] of the metric system. Particular emphasis is placed on those units used to indicate the size of cells and cell structures.

The features, their functions, and the use of the compound light microscope and the binocular dissecting microscopes are explained in detail. The scanning and transmission electron microscopes are explained in theory as their micrographs appear throughout these exercises. The compound light microscope is used to examine cells of 3 different organisms.

Metric System

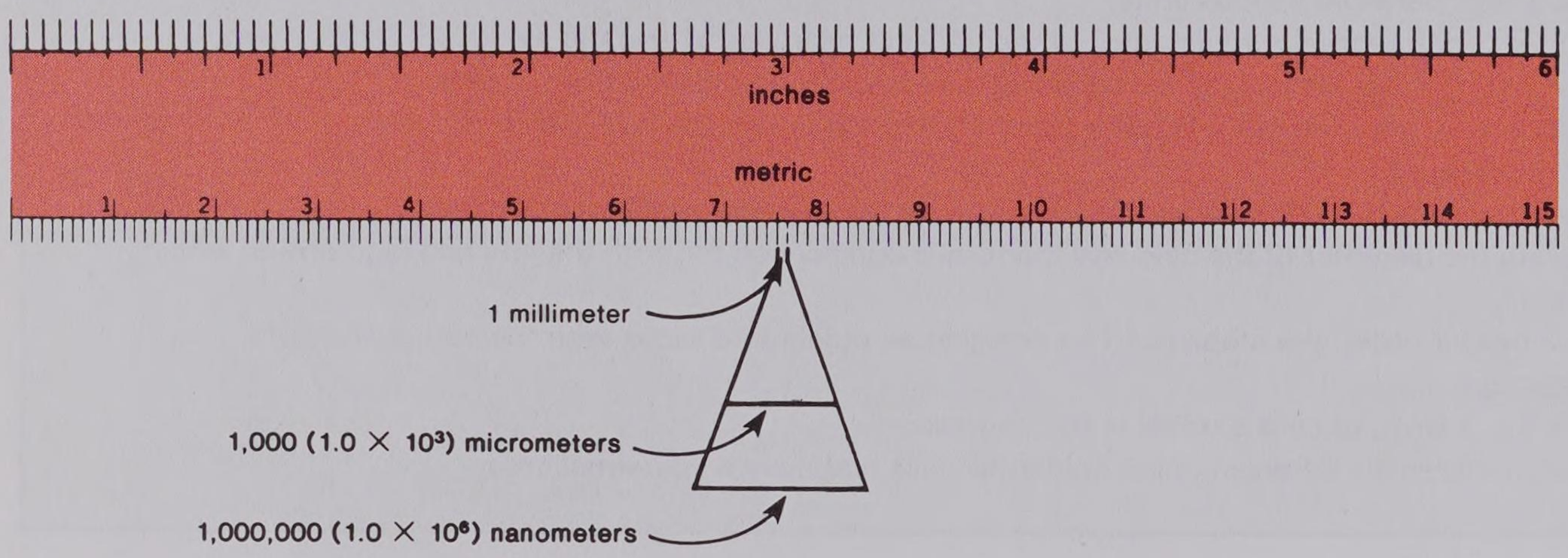
The metric system, because of its decimal organization, makes conversion from one kind of unit much easier than in the English system of measurement.

[1]Linear measurements only are considered in this laboratory. Other types of measurements are reviewed in appendix B.

Table 1.1 Metric Unit Values

Unit	Symbol	Value (m)	Value (cm)	Value (mm)	Value (μm)	Value (nm)
Meter	m	1.0 m	100 cm	1,000 mm	1,000,000 μm	1,000,000,000 nm
Centimeter	cm	0.01 m	1.0 cm	10 mm	10,000 μm	10,000,000 nm
Millimeter	mm	0.001 m	0.1 cm	1.0 mm	1,000 μm	1,000,000 nm
Micrometer	μm	0.000001 m	0.0001 cm	.001 mm	1.0 μm	1,000 nm
Nanometer	nm	0.000000001 m	0.0000001 cm	.000001 mm	0.001 μm	1.0 nm

Figure 1.1 Life-size ruler. The relationship of nanometers to micrometers to millimeters is indicated.



Conversions to a different size unit are made to avoid the use of extremely large and small numbers. Unit conversion can be made by simply moving the decimal to increase or decrease the size of the number.

Moving the decimal *left* results in *less* (the number is smaller).

Moving the decimal to the *right* results in *more* (the number is greater).

S 1. Using the number 3.4, move the *decimal* 3 spaces to the *left*. _____

Is this number less or more? _____

S 2. Using 3.4 again, move the *decimal* 3 spaces to the *right*. _____

Is this number less or more? _____

The metric unit selected is based upon the size of the object that is being measured. Here are some possible unit choices:

Classroom—meters (m) (approximately 39 inches)
Page size—centimeters (cm) (see ruler or fig. 1.1)
Cells and larger cell contents—micrometers (μm)
Smaller cell contents—nanometers (nm)

Table 1.1 indicates these relative sizes, and figure 1.1 graphically represents these units. Metric conversions can be made by using table 1.2 and the procedure described following.

Table 1.2 Metric Conversion

Unit	Value (decimal)	Value (fractional)	Value (exponent)	Relative Size	Conversion Direction	Decimal Direction
Meter (m)	1.0 m	1.0 m	10^1 m	Largest	More ↓ Less ↑	Right ↓ Left ↑
Centimeter (cm)	0.01 m	1/100 m	10^{-2}m			
Millimeter (mm)	0.001 m	1/1,000 m	10^{-3}m			
Micrometer (μm)	0.000001 m	1/1,000,000 m	10^{-6}m			
Nanometer (nm)	0.000000001 m	1/1,000,000,000 m	10^{-9}m	Smallest		

Conversion is obtained by taking the numerical *difference* between exponents and moving the decimal in the appropriate direction. As conversions are made, you should attempt to perceive the logical relationships. If the new unit is *smaller,* you need *more* of them as you move *down* the table and the decimal moves *right*. If the new unit is *larger,* you need *less* of them as you move *up* the table and the decimal moves *left*.

Example

Convert 3.1 centimeters (cm) into micrometers (μm).

Exponent for centimeters is	-2
Exponent for micrometers is	-6
Numerical difference (ignore sign) (as unit on a number line)	$(-)$ 4
Conversion direction	more (μm are smaller)
Move decimal	right (number is larger)

Therefore 3.1 cm = 31,000 μm

1. Use table 1.2 and the conversion procedure described to make the following *conversions*. Use figure 1.1 to aid in perceiving the logical relationships between these units and a metric ruler to make the measurements.

S 2. Obtain a ruler marked in centimeters and millimeters.

How many centimeters are in 1 in? _____

How many millimeters are in 1 cm? _____

To express the size of small objects, such as cell contents, biologists use even smaller units of the metric system than those on the ruler. These units are micrometers (μm) and nanometers (nm).

3. According to figure 1.1 and table 1.1, 1 mm = _____ μm = _____ nm. According to table 1.1, 1 μm = _____ mm and 1 nm = _____ μm.

To demonstrate that you understand the relationship of one metric unit to another, as described in figure 1.1 and table 1.1, fill in the blanks:

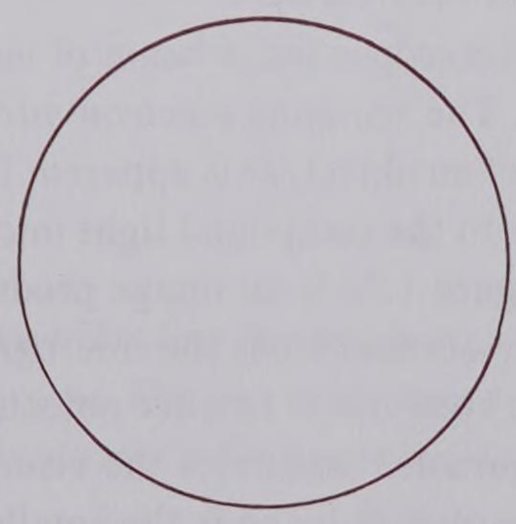
1.5 mm = _____ μm 1,000 nm = _____ μm

0.25 mm = _____ μm 1,500 μm = _____ mm

55 nm = _____ μm 7.5 μm = _____ nm

1.5 cm = _____ mm = _____ μm

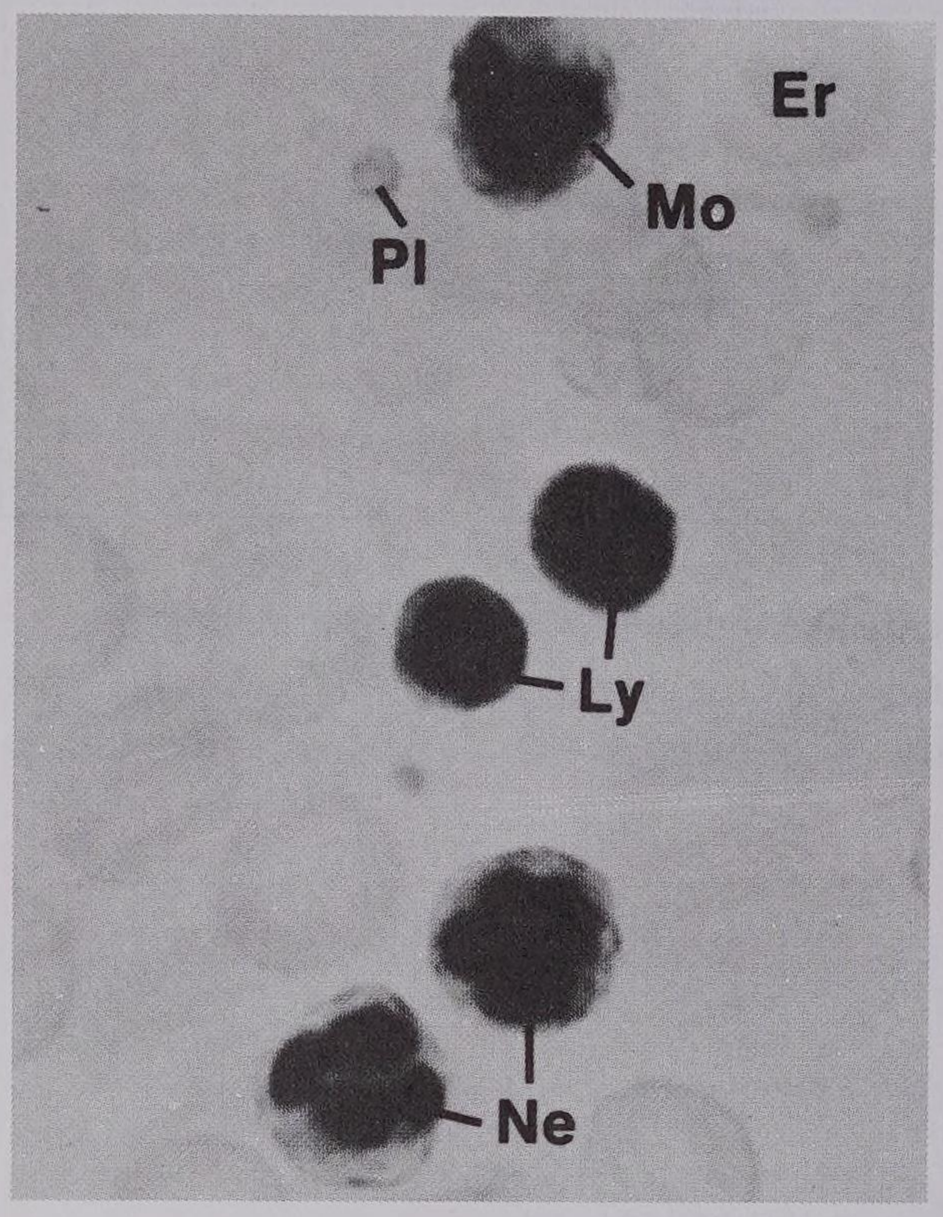
5,000 nm = _____ μm = _____ mm _____ cm



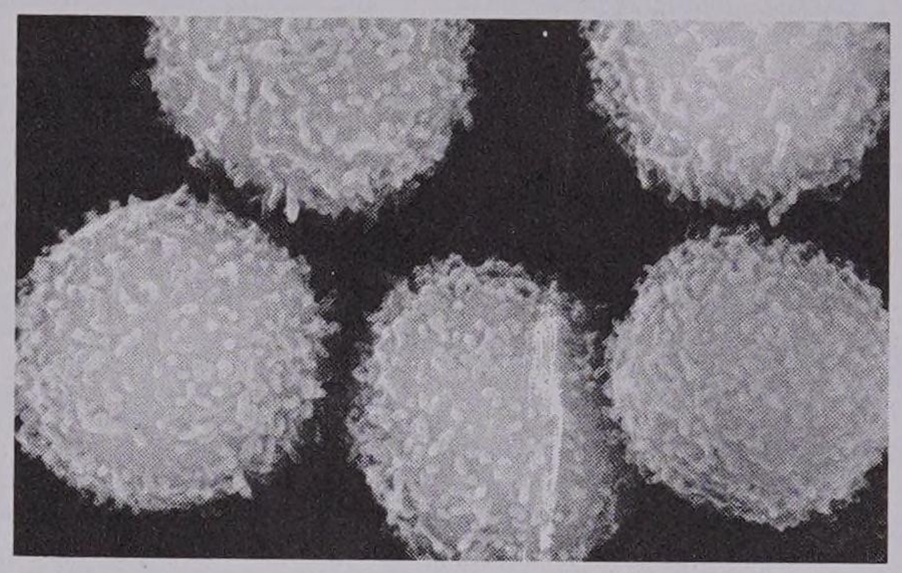
Measure the diameter of this circle to the nearest millimeter.

This circle is _____ mm; _____ μm; _____ nm.

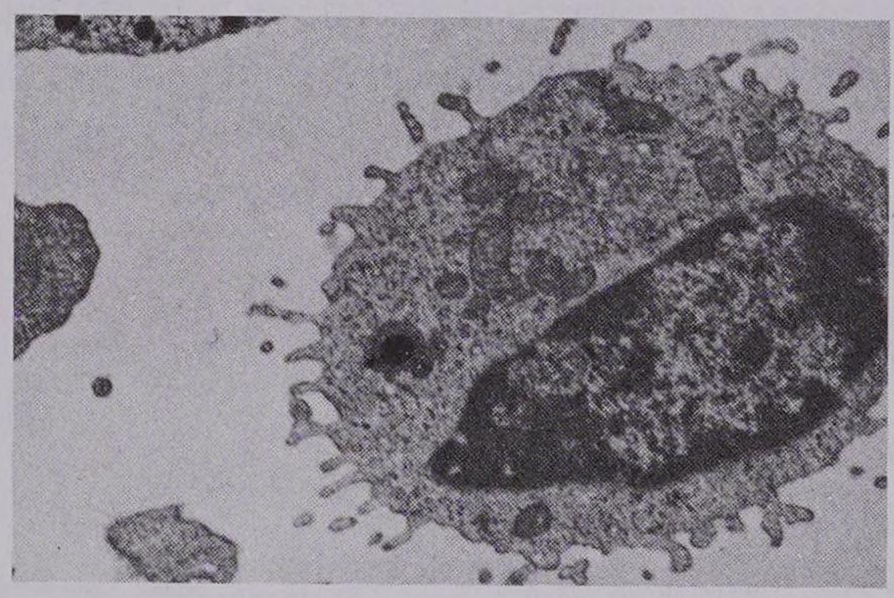
Figure 1.2 Images of a white blood cell, called a *lymphocyte,* produced by (***a***) compound light microscope, (***b***) scanning electron microscope, and (***c***) transmission electron microscope. Notice the greater amount of detail in (***c***) compared to (***a***). (***a:*** Courtesy Kessel, R. G. and Kardon, R. H., *Tissues and Organs: A Text-Atlas of Scanning Electron Microscopy.* © 1979 W. H. Freeman and Company)



a.



b.



c.

Microscopes

Because biological objects can be very small (micrometers or nanometers), it is often necessary to use a microscope to view them. Many kinds of instruments serve as effective magnifying devices, ranging from the hand lens to the electron microscope. A short description of 2 kinds of light microscopes and of the electron microscope follows. Your instructor may wish also to discuss the phase contrast, dark field, reflecting, polarizing, X-ray, fluorescent, and UV microscopes.

Light Microscope

Light microscopes use light rays that are magnified and focused by means of lenses. The *binocular dissecting microscope* is designed to study entire objects in 3 dimensions at low magnification. The *compound light microscope* is used for examining small or thinly sliced cross sections or longitudinal sections of objects under magnification that is higher than that of the binocular dissecting microscope. Illumination is from below, and the light passes through clear sections but does not pass through opaque sections. To improve contrast, the microscopist uses stains or dyes that bind to cellular structures and absorb light. Figure 1.2*a* is a photomicrograph, a photograph of an image produced by a light microscope.

Electron Microscope

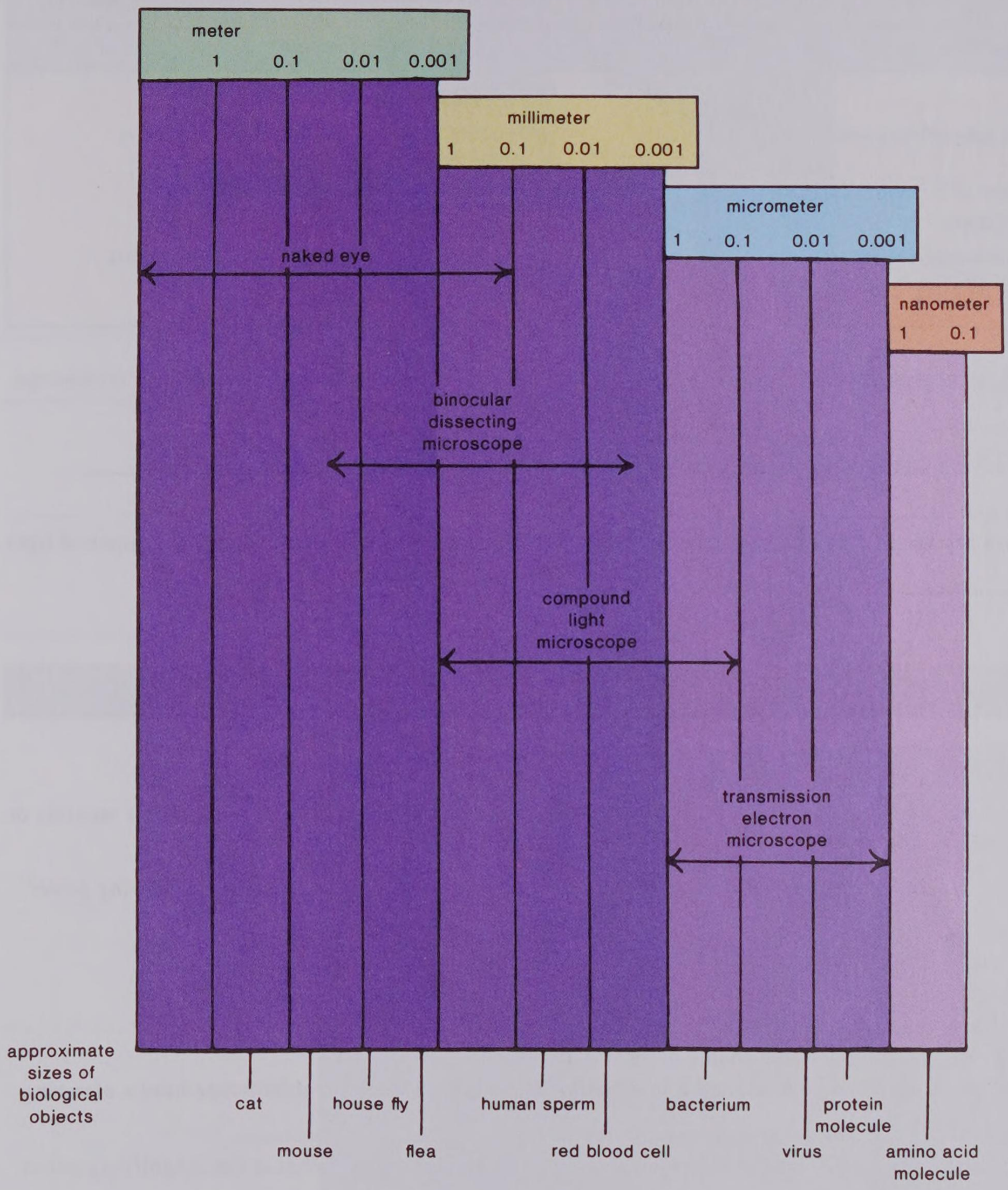
Electron microscopes use a beam of electrons that is magnified and focused on a photographic plate by means of electromagnets. The *scanning electron microscope* is analogous to the binocular dissecting microscope. It gives an image of the surface of an object, as is apparent from the electron micrograph in figure 1.2*b*. The *transmission electron microscope* is analogous to the compound light microscope. The object is thinly sliced and treated with heavy metal salts to improve contrast. Figure 1.2*c* is an image produced by this type of microscope.

It is obvious from the micrographs in figure 1.2 that both the scanning and transmission electron microscopes allow one to view much smaller objects, and with greater detail, than the compound light microscope.

Ⓢ Figure 1.3 compares the visual range of these microscopes to that of the naked eye. According to this figure, which of the objects listed is the smallest object that could be detected by the

1. eye? _____

Figure 1.3 Visual range of microscopes as compared to the range of the naked eye.



2. binocular dissecting microscope? _____

3. compound light microscope? _____

4. electron microscope? _____

The difference between these 2 instruments is not simply a matter of magnification; it also has to do with the electron microscope's ability to show detail, or the *resolving power* of the microscope. The use of electrons rather than light gives the electron microscope a much greater resolving power. Table 1.3 lists several differences between the compound light microscope and the transmission electron microscope.

Table 1.3 A Comparison of Light and Electron Microscopes

Light (Compound)	Electron (Transmission)
1. Glass lenses	1. Electromagnetic lenses
2. Illumination by light of many wavelengths	2. Illumination due to beam of electrons of one wavelength
3. Resolving power $\cong$ 0.1 μm	3. Resolving power $\cong$ 1 nm = 0.001 μm
4. Magnifies to 2,000×	4. Magnifies to 100,000×
5. Costs up to thousands of dollars	5. Costs up to hundreds of thousands of dollars
6. Specimen may be living or dead	6. Specimen must be dead

1. Using table 1.3, what is the resolving power (lower limit of the visual range) of the compound light microscope (in μm)? _____
2. What is the resolving power of the transmission electron microscope in (nm)? _____
3. Convert this to μm. _____ .
4. How many *times greater* is the resolving power of the transmission electron microscope than the compound light microscope? _____

Use of the Compound Light Microscope

Identification of Parts

$ After your instructor has told you how to carry the microscope, obtain one from the cabinet and place it securely on the table. Identify the following parts on your microscope and label fig. 1.4:

Eyepiece (ocular lens): Topmost series of lenses through which an object is viewed. What is the *magnifying power* of your ocular lens? _____

Body tube: Holds nosepiece at one end and eyepiece at the other end; conducts light rays.

Arm: Supports upper parts and provides carrying handle.

Nosepiece: Revolving device that holds objectives.

Objectives: (Individual objectives are not identifiable in fig. 1.4.)

Scanning power objective: Holds 4× lens used to view the whole slide. Does your microscope have a scanning power objective? _____ If yes, what is its magnifying power? _____

Low power objective: Holds 10× lens used to view the object in greater detail. What is the magnifying power of this lens on your microscope? _____

High power objective: Hold 40× (43× or 45×) lens used to view the object in even greater detail. What is the magnifying power of this lens on your microscope? _____

Oil immersion objective: (on microscopes with 4 objective lens) Holds a 95× (to 100×) lens and used in conjunction with immersion oil to view objects with the greatest magnification.

Does your microscope have an oil immersion objective? _____
If this lens is available, your instructor will discuss its use when it is needed.

Coarse adjustment knob: Knob used to bring object into approximate focus; used only with low power objective.

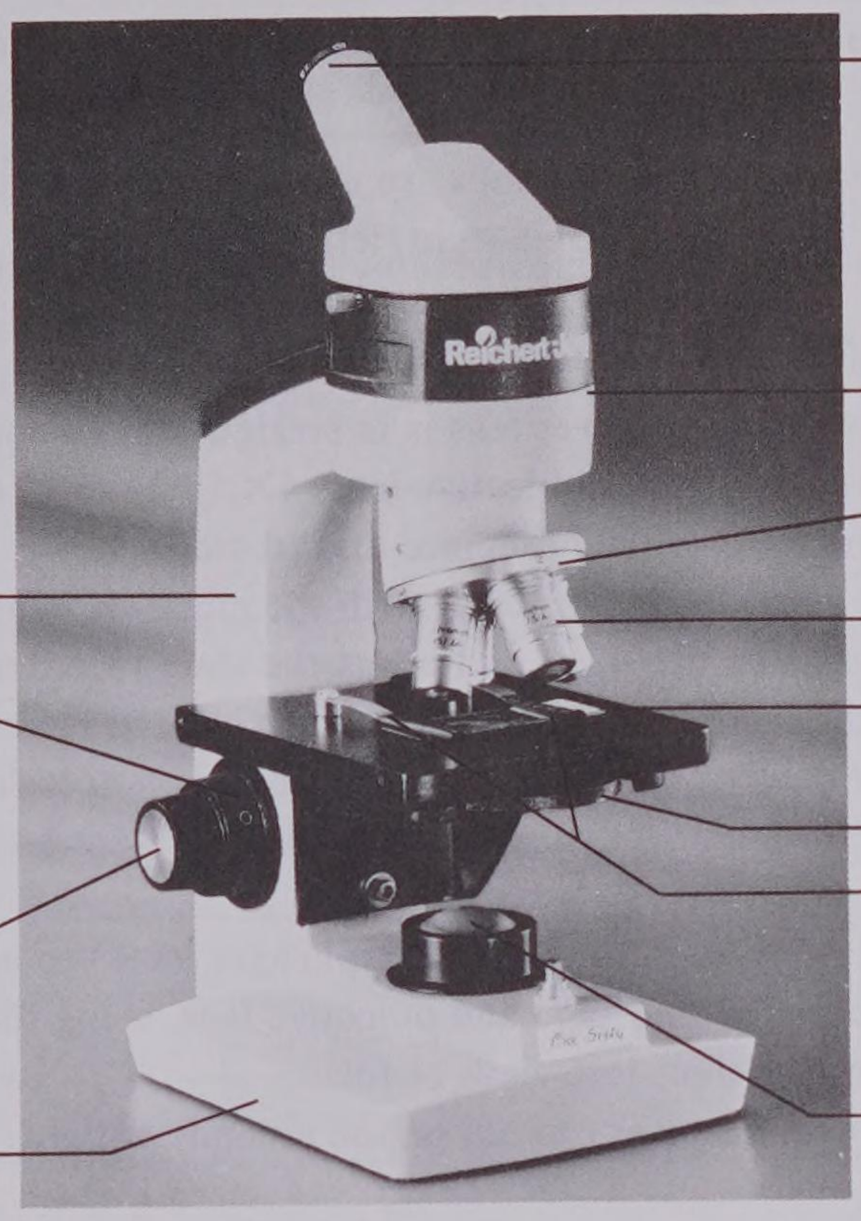
Fine adjustment knob: Knob used to bring object into final adjustment.

Diaphragm or Diaphragm control lever: Controls amount of illumination used to view the object.

Light source: An attached lamp that directs a beam of light up through the object.

Base: The flat surface of the microscope that rests on the table.

Figure 1.4 ***a.*** Components of a compound light microscope. ***b.*** Compound light microscope with binocular head and mechanical stage.



a.

a. _____

b. _____

c. _____

d. _____

e. _____

f. _____

g. _____

h. _____

i. _____

j. _____

k. _____

l. _____

b.

a. _____

b. _____

c. _____

d. _____

e. _____

f. _____

g. _____

h. _____

i. _____

j. _____

k. _____

l. _____

m. _____

Stage: Holds and supports microscope slides.
Stage clips: Holds slides in place on the stage.
Mechanical stage: (optional) A movable stage that aids in the accurate positioning of the slide.

Does your microscope have a mechanical stage? _____

Mechanical stage control knobs: (optional) These 2 knobs are usually located below the stage. One knob controls forward/reverse movement, and the other controls right/left movement.

Focusing the Microscope—Lowest Power

1. Turn the nosepiece such that the *lowest* power lens is in straight alignment over the stage.
2. Always begin focusing on the *lowest* power objective lens, 4× (scanning) or 10× (low power).
3. With the coarse adjustment knob, raise the nosepiece until it stops.
4. Place a slide of letter *e* on the stage, and stabilize it with the clips. (If your microscope has a mechanical stage, pinch the spring of the slide arms on the stage and insert the slide.) Center the *e* as best you can on the stage. (Use the 2 control knobs for the mechanical stage, which are located below the stage, to center the *e*.)
5. Again, be sure that the lowest power objective is in place; then as you look from the side, decrease the distance between the stage and the tip of the objective lens until it comes to an automatic stop or no closer than 3 mm above the slide.
6. Looking into the eyepiece, rotate the diaphragm (or diaphragm lever) to give the maximum amount of light.
7. Slowly increase the distance between the stage and objective lens, using the coarse adjustment knob, until the object—in this case, the letter *e*—comes into view, or focus.
8. Once the object is seen, it may be necessary to adjust the amount of light. To increase or decrease the contrast, rotate the diaphragm slightly.
9. Use the fine adjustment knob to sharpen the focus if necessary.
10. Practice having both eyes open when looking through the eyepiece, as it greatly reduces eyestrain.

Inversion

1. In the space provided here, draw the letter *e* as it appears on the slide (look from the side, not through the eyepiece).
2. Next to this, draw the letter *e* as it appears when you look through the eyepiece.

1.	2.

3. What differences do you notice? _____ Inversion refers to the fact that the image is not only inverted but also reversed. Move the slide to the right. Which way does the image appear to move? _____

Focusing the Microscope—Higher Powers

Compound light microscopes are *parfocal;* that is, once the object is in focus with lowest power, it should also be almost in focus with the higher power.

1. Bring the object into focus under the lowest power by following instructions in the previous section.
2. Make sure the letter *e* is centered in the field of the lowest objective.
3. Move to the next higher objective (low power [10×] or high power [45×]) by turning the nosepiece until you hear it click into place. Do not change the focus; parfocal microscope objectives will not hit the slide when changing the focus if the lowest objective is initially in focus. (If you are on low power [10×], proceed to high power [45×] before going on to step 4.)
4. If any adjustment is needed, use only the *fine* adjustment knob. Always use only the fine adjustment knob with high power. On your drawing of the letter *e* as it was observed initially, *draw* a circle around the portion of the letter that you are now seeing with high power magnification.

5. When you have finished your observations of this slide (or any slide), rotate the nosepiece until the lowest power objective clicks into place, and then remove the slide.
6. The following rules should be observed:
   a. The lowest power, scanning or low, objective should be in position both at the beginning and end of use.
   b. Use only lens paper for cleaning lenses.
   c. Do not tilt the microscope when viewing a wet mount.
   d. Keep the stage clean and dry to prevent rust and corrosion.
   e. Do not remove parts of the microscope.
   f. Keep the microscope dust free by covering.
   g. Report any malfunctioning.

Total Magnification

Total magnification is calculated by multiplying the magnification of the ocular lens by the magnification of the objective lens.

What is the total magnification of the following:

	Ocular Lens		Objective Lens		Total Magnification
Scanning power (if present)	_____	×	_____	=	_____
Low power	10	×	10	=	100
High power	10	×	40	=	400
Oil immersion (if present)	_____	×	_____	=	_____

Diameter of Field

Low Power

1. Place a clear plastic ruler across the stage so that the edge of the ruler is visible as a horizontal line along the diameter of the low power (10×) (not scanning) field. Be sure that you are looking at the millimeter side of the ruler.
2. Estimate the number of millimeters, to tenths, that you see along the field and record them here: 2 .
   Change the figure to micrometers (μm) 2000 . This is the diameter for your low power (10×) field.

High Power

Place the data for your specific microscope into the following spaces:

LPD = low power diameter of field (in μm) 2000

LPM = low power magnification (not scanning) 100

HPM = high power magnification 400

To compute the high power diameter of field (HPD), insert the preceding data into the following formula:

$$HPD = LDP \times \frac{LPM}{HPM}$$

$$HPD = (2000) \times \frac{(100)}{(400)} = 500$$

In comparing low power to high power, which has a larger field and allows you to see more of the object? _____

Which has a smaller field but magnifies to a greater extent? _____

To locate small objects that are seen on low power, they must be in the *center* of the field before rotating to high power.

Depth of Focus

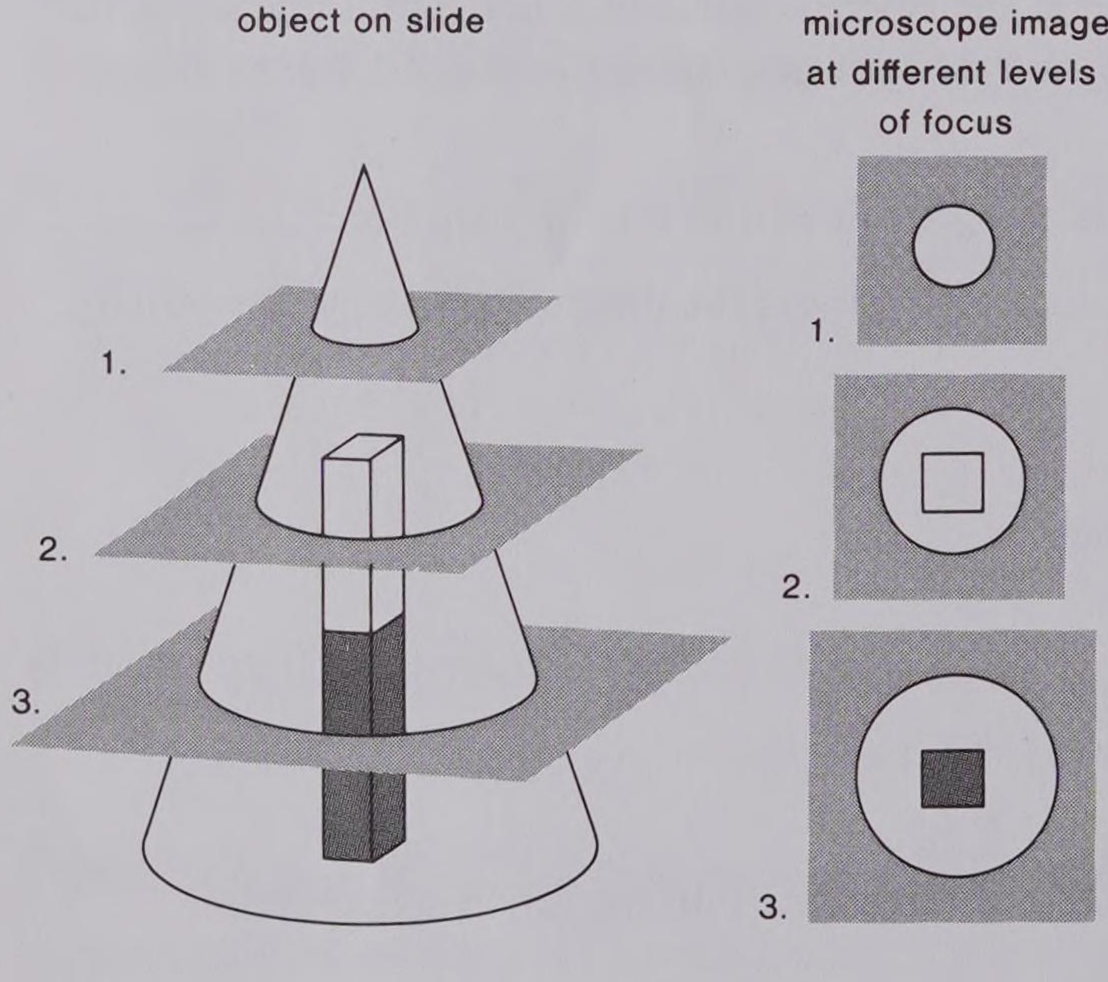
1. Obtain a prepared slide with 3 or 4 colored threads mounted together, or prepare a wet mount slide (using the directions that follow) by crossing threads or hair of different colors.
2. With low power, find a point where the threads or hairs cross. Slowly focus up and down. Notice that when one thread or hair is in focus, the other seems blurred. Determine the order of the threads or hairs and complete table 1.4.

Table 1.4 Order of Threads (or Hair)

Depth	Thread (or Hair) Color
Top	
Middle	
Bottom	

The vertical distance that remains in focus at one time is called the *depth of focus*. Switch to high power and notice that the depth of focus is more shallow with high power than with low power. If you make constant use of the fine adjustment knob when viewing a slide with high power, you will get an idea of the specimen's 3-dimensional form. It would be possible, for example, to reconstruct the 3-dimensional structure in figure 1.5 from sections 1, 2, and 3 in the figure.

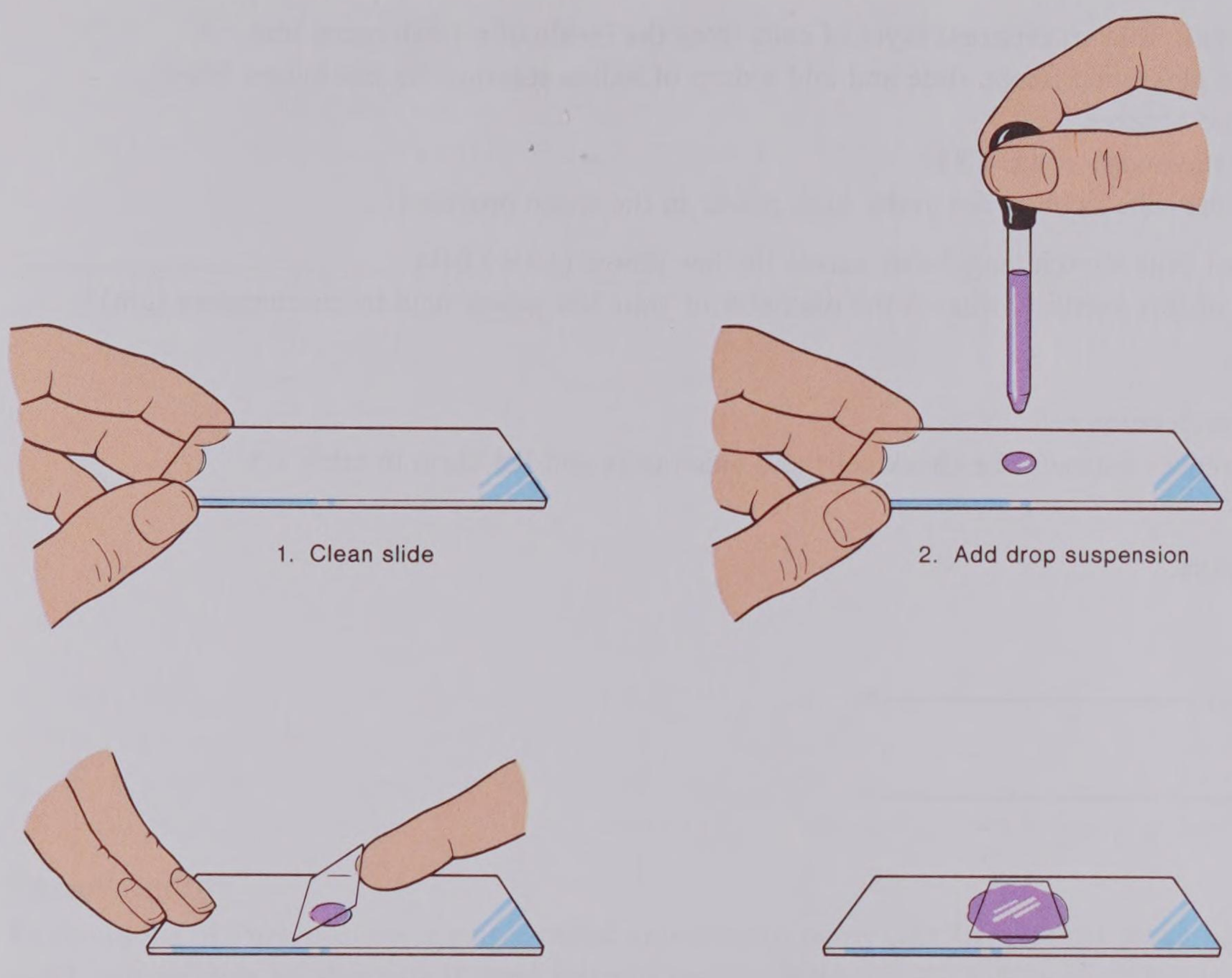
Figure 1.5 A demonstration of how focusing at depths (1), (2), and (3) would produce 3 different images that could be used to reconstruct the original 3-dimensional structure.



Observations

Often it is necessary to prepare a specimen for observation. In such cases, the object should always be viewed as a *wet mount*. A wet mount is prepared by placing a drop of liquid on a slide or, if the material is dry, by placing it directly on the slide and adding a drop of water or stain. The mount is then covered with a *coverslip,* as illustrated in figure 1.6.

Figure 1.6 Preparation of wet mount.

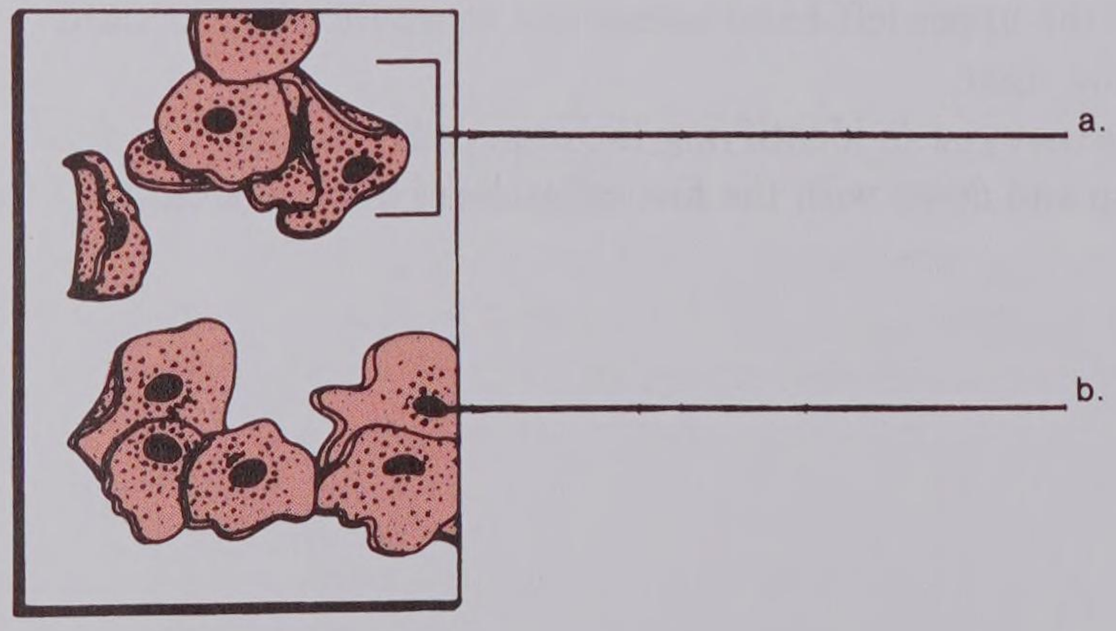


To get more experience preparing slides and using the compound light microscope, you will now look at 2 types of epidermal cells, the outer layer of cells covering the body of plants and animals.

Human Epidermal Cells

1. Obtain a prepackaged flat toothpick (or sanitize one with the alcohol or alcohol swabs provided).
2. Gently scrape the inside of your cheek with the toothpick and place the scrapings on a clean, dry slide.
3. Add a drop of very weak *methylene blue* or *iodine* solution, and cover with a coverslip.
4. Observe under the microscope.
5. Locate the nucleus, the central round body in each cell (see fig. 1.7).
6. Draw to scale and label the cell, as observed under high power, in the space provided. The granular specks are other organelles, which will be discussed in another exercise.

Figure 1.7 Human epidermal cells (cheek).



sketch

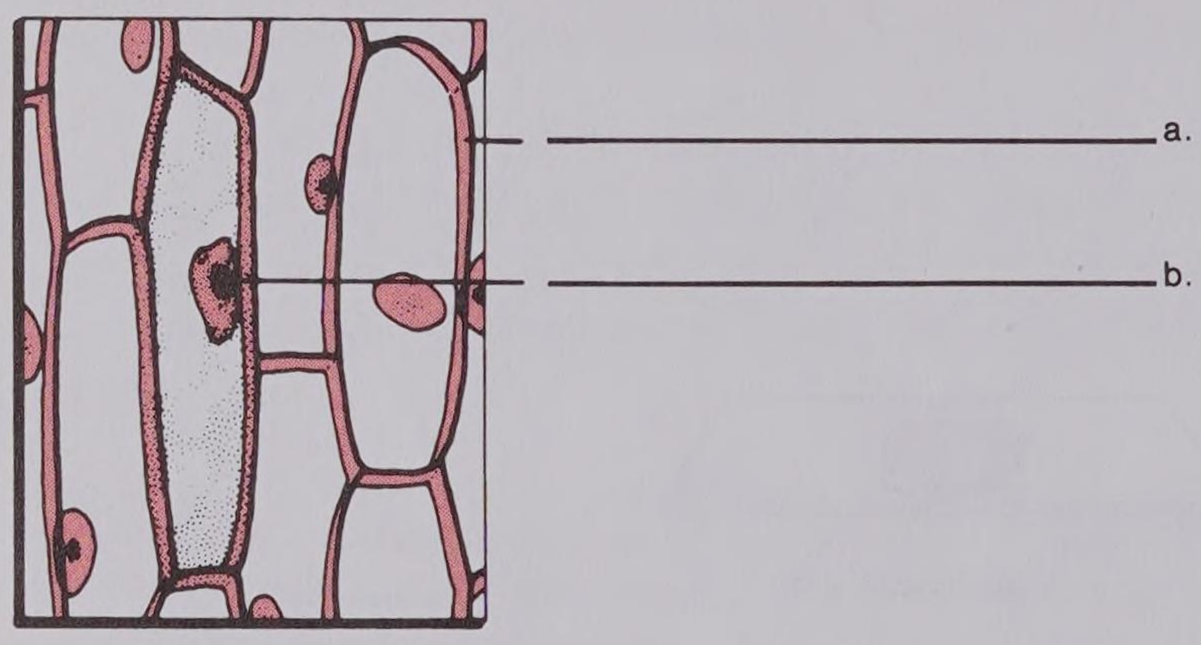
Onion Epidermal Cells

S 1. With a scalpel, strip a small, thin transparent layer of cells from the inside of a fresh onion leaf.
S 2. Place it gently on a clean glass microscope slide and add a drop of iodine solution (or *methylene blue*).
S 3. Cover with a coverslip and observe.
S 4. Locate the cell wall and the nucleus (fig. 1.8).
S 5. Draw to scale and label the cells as observed under high power in the space provided.
S 6. Estimate how many onion cells stretch lengthwise across the low power (10×) field. _____
From a previous section of this exercise, what is the diameter of your low power field in micrometers (μm)?

_____ .

Calculate the length of each onion cell. _____
S 7. Note some obvious differences between the cheek cells and onion cells and list them in table 1.5.

Figure 1.8 Onion epidermal cells.



sketch

Table 1.5 Differences between Human and Onion Epidermal Cells		
Differences	**Human Epidermal Cells (Cheek)**	**Onion Epidermal Cells**
Shape		
Cell wall		

Pond Water

Examination of pond water serves as a critical test of your ability to observe objects with the microscope, to utilize depth of focus, and to control illumination to heighten contrast.

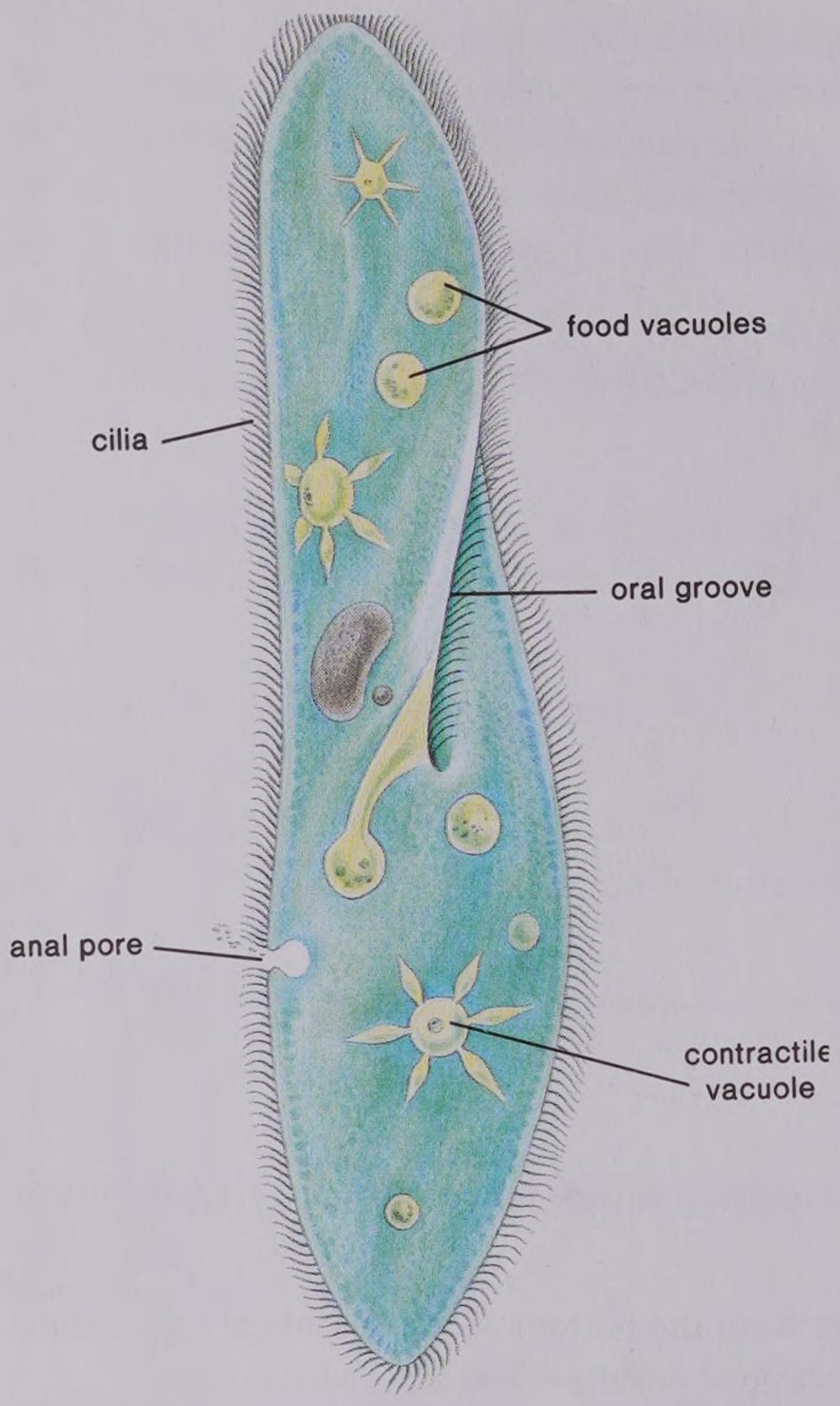
S 1. Make a wet mount of pond water by taking a drop from the bottom of the container.
S 2. To locate any organisms present, scan the slide: start at the upper left-hand corner and move the slide forward and back as you work across the slide from the left to the right.
S 3. Use the pictorial guides provided by your instructor to assist you in identifying the organisms present.
S 4. Experiment using *all* available objective lenses, focus up and down with the fine adjustment knob, and adjust the light so that it is not too bright.
S 5. Draw to scale, and label in the space provided.

Paramecium

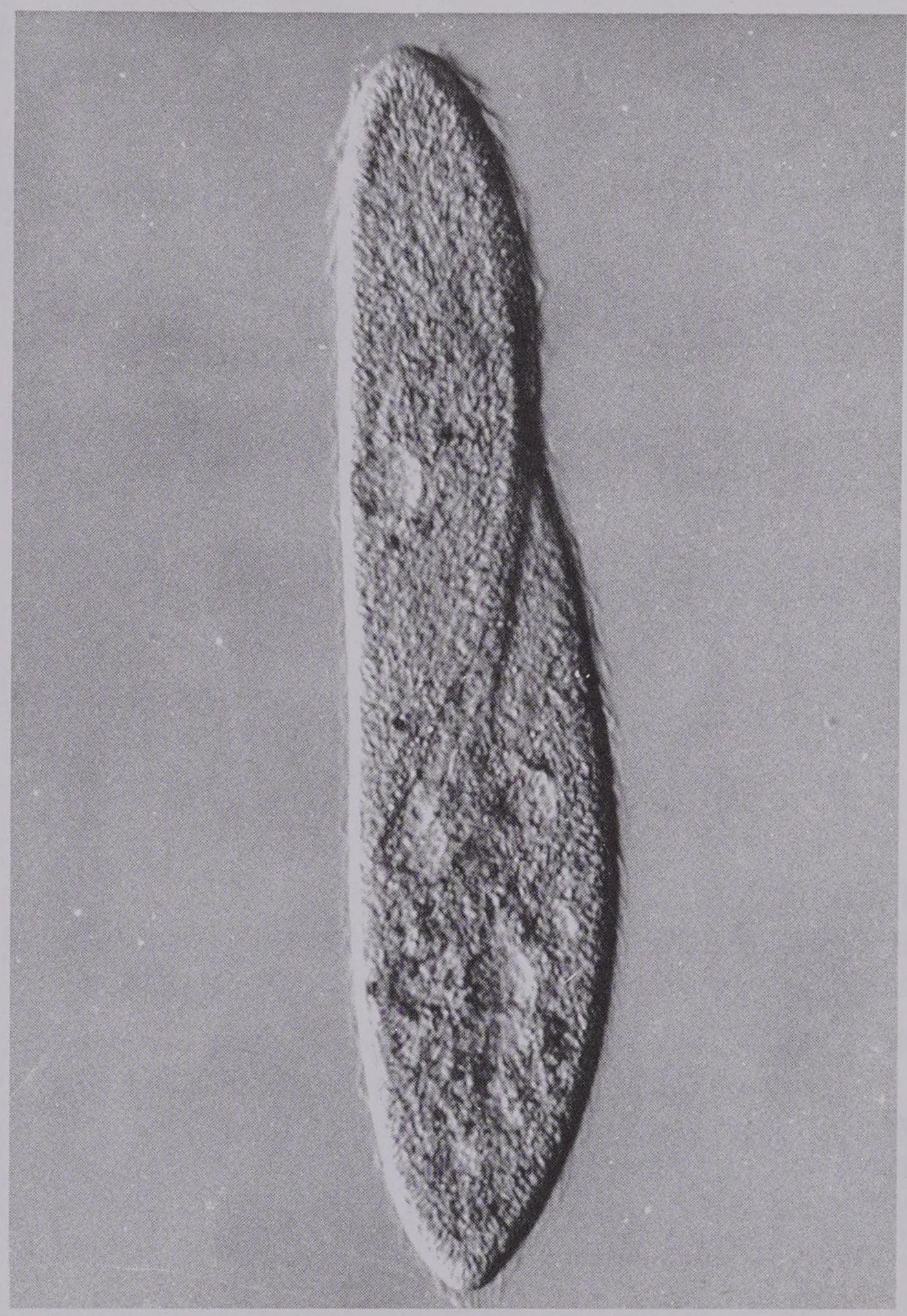
Examination of *Paramecium,* a single-celled animal with many cilia to facilitate movement (or any other available culture), can serve as an alternate if pond water is not available.

1. Make a wet mount of *Paramecium* by using a drop of the culture from the bottom of the container and adding a drop of *protoslo* (*methyl cellulose*) solution on a slide. The protoslo slows the organism's swimming.
2. Mix thoroughly, and add a coverslip.
3. To locate any *Paramecium* present, scan the slide: start at the upper left-hand corner and move the slide forward and back as you work across the slide from the left to the right. The *Paramecium* may be at the edge of the slide because it shows an aversion to the protoslo. Use the figure 1.9 to help you identify the structural details.
4. Experiment using *all* available objective lenses, focus up and down with the fine adjustment knob, and adjust the light so that it is not too bright.
5. Draw to scale, and label in the space provided.
6. You are now finished with the compound light microscope. Turn the nosepiece to the lowest power objective, and be sure the stage is dry and clean.
7. Return your microscope to its correct location in the cabinet.

Figure 1.9 ***a.*** Diagrammatic representation of *Paramecium*. ***b:*** Photomicrograph of *Paramecium*.



a.



b.

Binocular Dissecting Microscope (Stereomicroscope)

Binocular dissecting microscopes are used for viewing objects in 3 dimensions at low magnifications. They are used to study entire small organisms, any object requiring lower magnification, and opaque objects that can only be viewed by reflected light.

Ⓢ 1. Obtain a binocular dissecting microscope and a separate illuminator, if necessary, from the cabinet.

Ⓢ 2. Plug in the power cord and turn on the illuminator. There is a wide variety of binocular dissecting microscope styles, and your instructor will discuss the specific style(s) available. Regardless of style, the following features should be present.

Ⓢ 3. Locate each of these parts on your binocular dissecting microscope, and label them on figure 1.10.

Binocular head: Holds 2 eyepiece lenses that are moveable to accommodate for the various distances between different individuals' eyes.

Eyepiece lenses: Two eyepiece lenses are located on the binocular head.

What is the magnification of your eyepieces? _____

Some models have one independent focusing eyepiece with a knurled knob to allow independent adjustment of each eye.

Figure 1.10 Binocular dissecting microscope (stereomicroscope).

Does your microscope have an independent focusing eyepiece? _____

The nonadjustable eyepiece is called the fixed eyepiece.

Focusing knob: A large black knob located on the arm; used for changing focus of both eyepieces together.

Magnification changing knob: A knob below the binocular head that is used to change magnification of both eyepieces simultaneously. This may be a zoom mechanism or a rotating mechanism with fixed lenses of different power that click into place.

Focusing the Binocular Dissecting Microscope

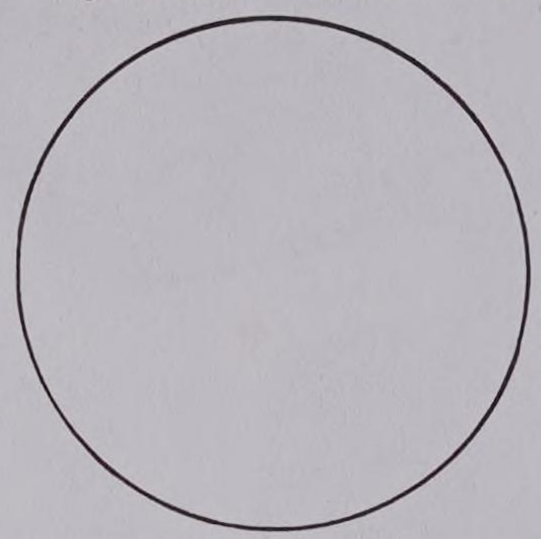
1. Place any kind of 3-dimensional object in the center of the stage.
2. Adjust the distance between the eyepieces on the binocular head so that they comfortably fit the distance between your eyes. You should be able to see the object in both eyes as one 3-dimensional image.
3. Bring the object into focus using the focusing knob.
4. Does your microscope have an independent focusing eyepiece? __ If so, use the focusing knob to bring the image in the fixed eyepiece into focus. Then adjust the independent focusing eyepiece so that the image is clear.

Is the image inverted? _____

5. Turn the magnification changing knob and determine the kind of mechanism on your scope. A zoom mechanism gives continuous view while changing the magnification. A rotating lenses mechanism will block the view of the object as the new lenses are rotated. Be sure to click them firmly into place. If the field is only partially visible, they are not clicked totally into place.

   What kind of mechanism is on your scope? _____

6. Set the magnification changing knob on the lowest magnification. Sketch the object in the following circle as though this represents your entire field of view.



7. Rotate the magnification changing knob to the highest magnification. Draw another circle within the one provided to indicate the reduction of the field of view.
8. Experiment with various objects at various magnifications until you become comfortable with the use of the binocular dissecting microscope.
9. When you are finished, return your binocular dissecting microscope and illuminator to their correct positions in the cabinet.

Laboratory Review 1

_____ 1. 1.5 cm equals how many mm?

_____ 2. 15 mm equals how many μm?

_____ 3. 20 nm equals how many μm?

_____ 4. Your lab partner is looking at a slide and the objective is almost touching the coverslip on the slide. What objective is he or she using?

_____ 5. If the ocular lens in use is 10×, what objective lens will give you a total magnification of 100×?

_____ 6. What part of the microscope is used to adjust the amount of light coming through the lenses?

_____ 7. If the diameter of the field is 1.6 mm and you count 40 consecutive cells from one end of the field to another, how wide is each cell in micrometers (μm)?

_____ 8. What type of microscope, aside from the compound light microscope, might you use to observe the organism found in a sample of pond water?

_____ 9. What are 2 differences between onion epidermal cells and human epidermal cells?

_____ 10. The letter *e* is entirely visible when using the scanning lens, but when using the high power lens, you cannot see the entire letter. Explain the difference.

11. Briefly describe the necessary steps to observe a slide under the microscope using low power.

References

Jakowska, S. 1973. *SICC Laboratory Manual for Biology 101.* Dubuque, IA: Kendall/Hunt Publishing Company.

Paul, C. 1972. *Principles of Biological Science.* Dubuque, IA: Kendall/Hunt Publishing Company.

Laboratory

2

Chemical Composition of Cells

Learning Objectives

Students should be able to

1. name the organic chemical components of cells studied in this lab and be able to recognize their chemical structure;
2. cite the test used to identify the presence of proteins (and polypeptides), starch, sugars (reducing), and lipids;
3. distinguish test responses that show a chemical's absence (negative) or presence (positive);
4. describe the variable response of the Benedict's test.

Introduction

In this laboratory you study proteins, carbohydrates, and fats—common organic molecules found in cytoplasm, the matrix of the cell. Large organic molecules often contain small unit molecules joined in a set sequence. As we shall see, proteins contain amino acids; starch (a carbohydrate) contains glucose; and a neutral fat contains glycerol and fatty acids.

Various chemicals will be used to test for the presence of these molecules. Most often we will be looking for a color change. If the change is observed, it is said that the test is *positive* because it indicates that a particular molecule is present. If the change in color is not observed, it is said that the test is *negative* because it indicates that a particular molecule is not present.

In all of the procedures that you perform, you need to include a distilled water sample. This is called a *control* and is essential to the experiment. Usually the control will be a replicate of the experiment, lacking one essential factor (the *experimental variable*). With this factor missing, you are able to observe the difference between a positive and negative result. If the control sample should happen to test positive, you know your test is invalid.

Safety in the Laboratory

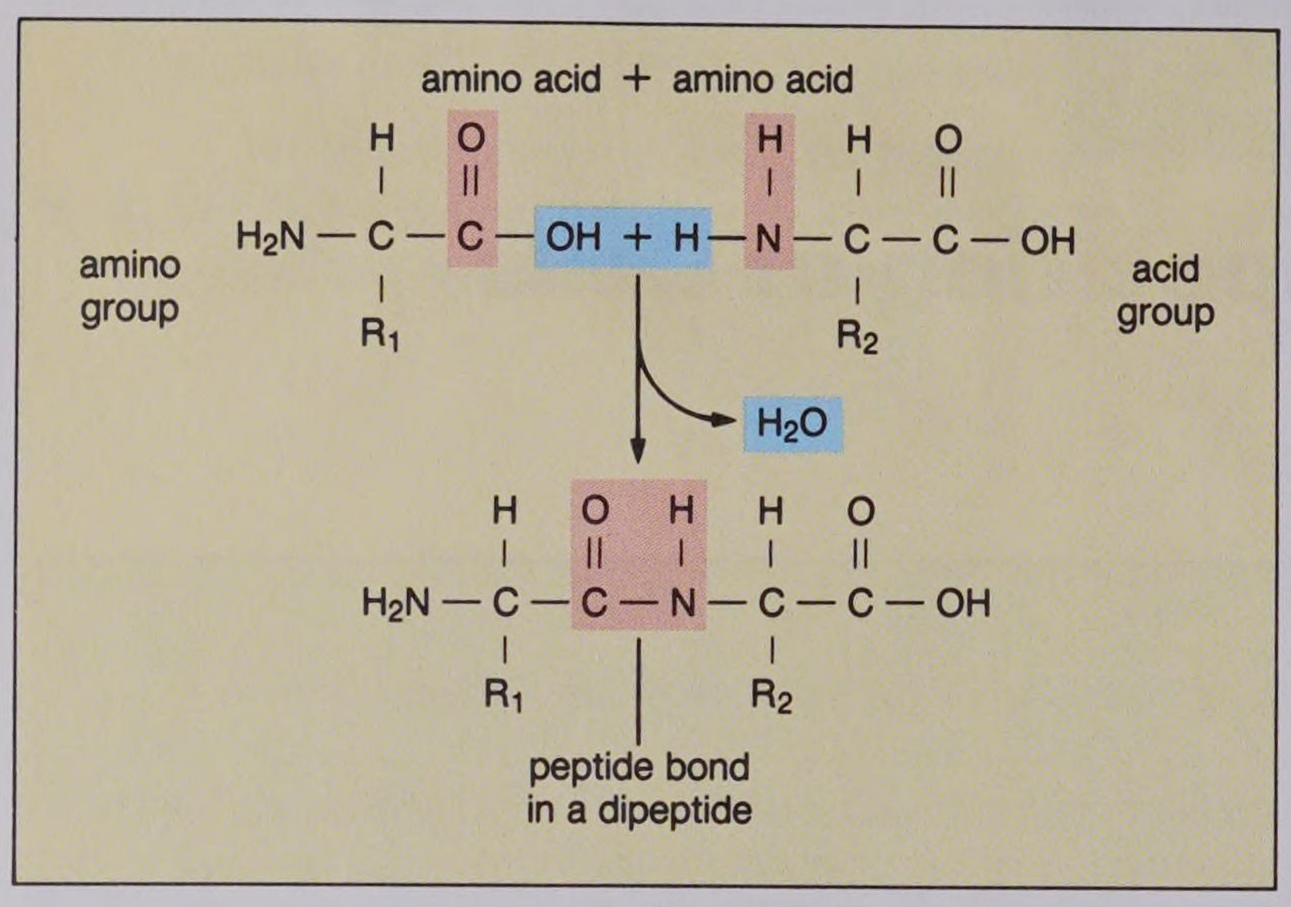
When working with chemicals, glass test tubes, hot water baths, and so forth, it is necessary to follow safety precautions. Your particular school may have set regulations that your instructor will explain to you. Your instructor will also tell you what to do should any unforeseen circumstance occur.

Proteins

Protein Structure

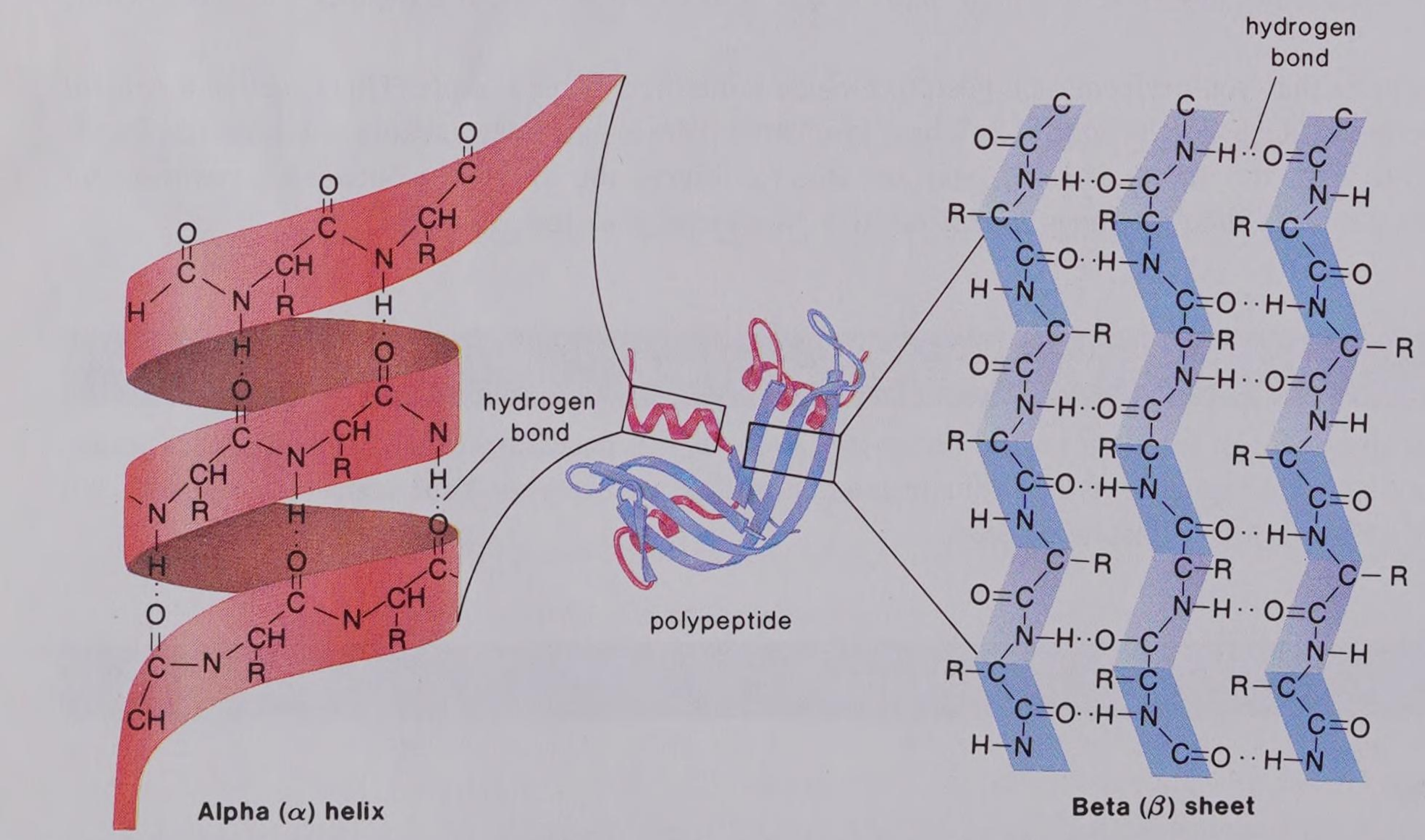
Proteins are gigantic organic molecules composed of *polypeptide chains* made up of *amino acids*. There are about 20 different common amino acids in cells. All amino acids have an organic acid (COOH) and an amine (NH_2) portion (see fig. 2.1). They differ by the nature of their R (remainder) group. The R groups have varying sizes, shapes, chemical structures, and chemical activities.

Figure 2.1 Peptide bond formation between amino acids creates a dipeptide. Multiple amino acids forming peptide bonds establish the *primary structure* of a protein.



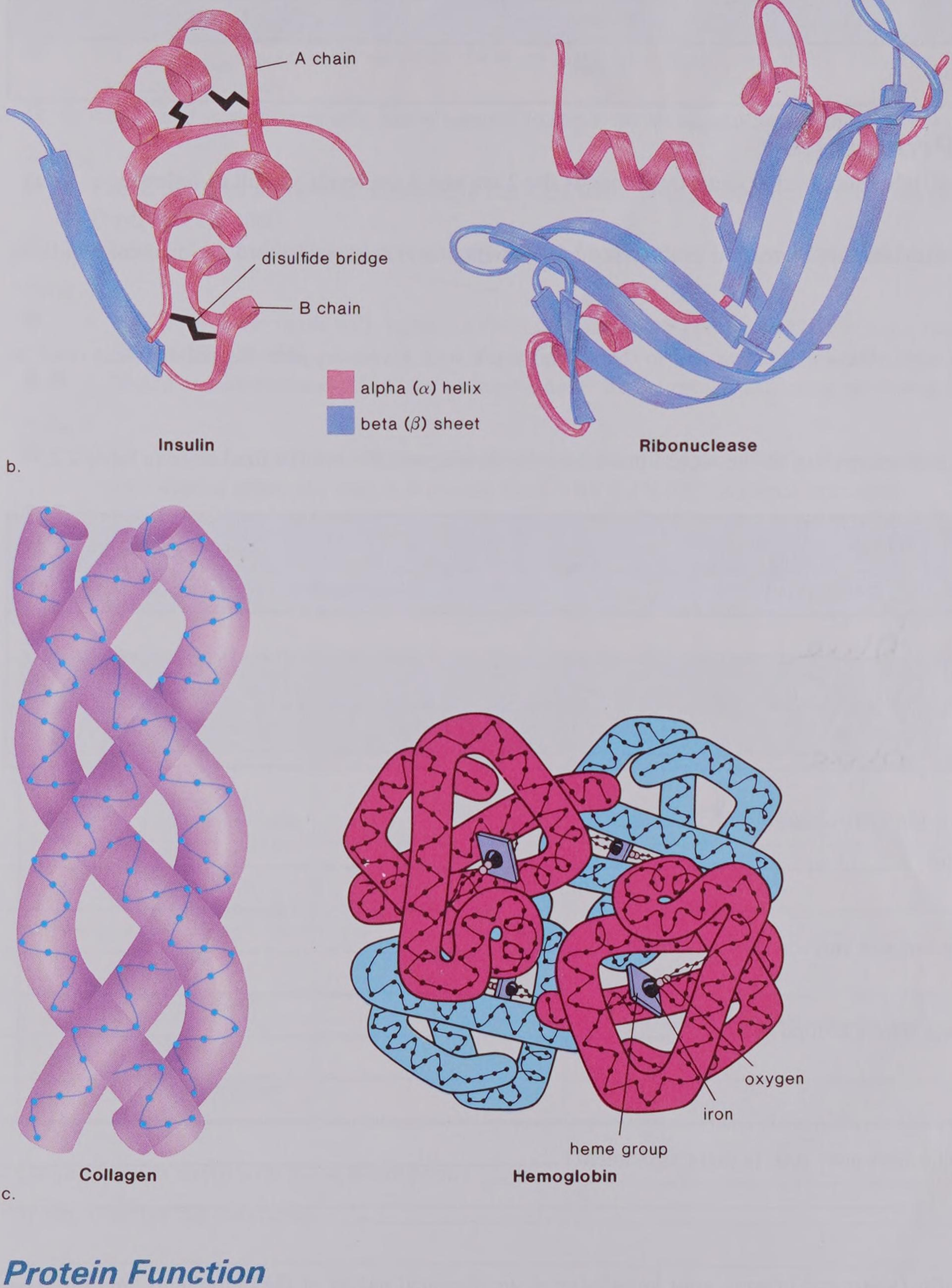
The *peptide* is a chain of several amino acids. The larger chains are called polypeptides. Amino acids are held together by peptide bonds —C(=O)—N(H)— formed between adjacent amine and acid groups (see figs 2.1 and 2.2*b*). In a protein, the polypeptide chains (100s of amino acids) form hydrogen bonds between adjacent amino acids. This form bends and folds to produce a 3-dimensional shape (see figs 2.2, 2.3, and 2.4). Some proteins have more than one polypeptide chain.

Figure 2.2 ***a.*** The *secondary* structure of polypeptides is dependent upon hydrogen bonding (dotted lines). ***b.*** The *tertiary* structure of polypeptides. ***c.*** Quaternary structure of some representative proteins.



a.

Figure 2.2 Continued



Protein Function

Proteins have numerous functions in cells. One important category of proteins are *enzymes,* organic catalysts that speed up metabolic reactions. In addition, the contractile elements of muscle cells contain protein filaments. *Albumin,* the protein that you will use in the next 2 experiments, is the major protein found in blood.

Test for Proteins

Biuret reagent (blue color) contains a strong solution of sodium or potassium hydroxide (NaOH or KOH) and a very small amount of very dilute copper sulfate ($Cu^{++}SO_4^{=}$) solution. The reagent changes color in the presence of a protein and peptides because the amino group ($—NH_2$) complexes with the copper ions (see table 2.1).

Table 2.1 Test for Protein		
	Protein	**Peptides**
Biuret reagent	Violet	Pinkish

Experimental Procedure

❸ With a marking pencil, label and mark 3 clean test tubes at the 2 cm and 4 cm levels and fill as follows:

Tube 1

❸ Fill to the first mark with *distilled water* and to the second mark with *biuret reagent*. Record the final color in table 2.2.

Tube 2

❸ Fill to the first mark with *albumin solution* and to the second mark with *biuret reagent*. Record the final color in table 2.2.

Tube 3

❸ Fill to the first mark with *starch* and to the second mark with *biuret reagent*. Record the final color in table 2.2.

Table 2.2 Biuret Test			
Tube No.	**Content**	**Color (final)**	**Conclusions**
1	Water	Blue	
2	Albumin	Purple	protein present
3	Starch	blue	

Which of these 3 tubes is the control sample? #1

What is a control sample? _____

_____

Why do experimental procedures include control samples? _____

_____

Is your data similar to the others around you? _____

Describe any difference. _____

_____

Discuss your results with others near you. Is there agreement? _____

Note any differences. _____

Conclusion

Using the preceding results (yours and others), your knowledge of the chemical nature of the ingredients, and the specificity of chemical tests, formulate a conclusion that includes the kind of chemical present (or absent) and why results occurred. Write these conclusions in Table 2.2.

Enzyme Function of Proteins

The enzyme *pepsin* occurs in the hydrochloric acid (HCl) environment of the stomach. In the experiment that follows, we test the ability of this enzyme to speed up the following reaction:

$$\text{protein} + \text{water} \xrightarrow[\text{(hydrochloric acid HCl)}]{\text{pepsin (enzyme)}} \text{peptides}$$

Experimental Procedure

With a wax pencil, label and mark 4 clean tubes at the 2 cm, 4 cm, 6 cm, and 8 cm levels, and fill and incubate as follows:

Tube 1

S 1. Fill to the 2 cm mark with *water,* to the 4 cm mark with *pepsin,* and to the 6 cm mark with *0.2% HCl* (hydrochloric acid).

S 2. Shake and immediately add *biuret reagent* to the 8 cm mark, and record the final color in table 2.3.

Tube 2

S 1. Fill to the 2 cm mark with *albumin,* to the 4 cm mark with *pepsin,* and to the 6 cm mark with *0.2% HCl* (hydrochloric acid).

S 2. Shake and immediately add *biuret reagent* to the 8 cm mark, and record the final color in table 2.3.

Tube 3

S 1. Fill to the 2 cm mark with *water,* to the 4 cm mark with *pepsin,* and to the 6 cm mark with *0.2% HCl* (hydrochloric acid).

S T 2. Shake and incubate at 37° C for at least 1 hour. Better results will occur with even longer incubation.

Tube 4

S 1. Fill to the 2 cm mark with (the protein) *albumin,* to the 4 cm mark with *pepsin* (a protein that is an enzyme that digests proteins), and to the 6 cm mark with *0.2% HCl* (hydrochloric acid).

S T 2. Shake and incubate at 37° C for at least 1 hour.

S When incubation is complete, fill tubes 3 and 4 to the 8 cm mark with *biuret reagent*. Note any color change and record your results in table 2.3.

Table 2.3 Action of Pepsin

Tube No.	Contents	Color (final)	Conclusions
1	Water Pepsin HCl (immediate)		
2	Albumin Pepsin HCl (immediate)		
3	Water Pepsin HCl (incubate)		
4	Albumin Pepsin HCl (incubate)		

Do your results agree with those around you? _____

If not, describe the differences. _____

_____

Conclusion

Evaluate the results obtained, and write your conclusions in table 2.3. Enzymes are specific and speed up only one type of chemical reaction. Considering this, do you predict that pepsin will break down starch? _____

Why or why not? _____

_____

This prediction is considered again later in this exercise.

Carbohydrates

Carbohydrates include large macromolecular molecules like *starch* and the simple *sugars*. *Glucose* has only one sugar unit and is a monosaccharide (fig. 2.3); *maltose* has 2 sugar units and is a disaccharide (fig. 2.4). Starch is a polysaccharide, a chain of glucose units (fig. 2.5).

Figure 2.3 Structure of glucose, a monosaccharide having 6 carbon atoms.

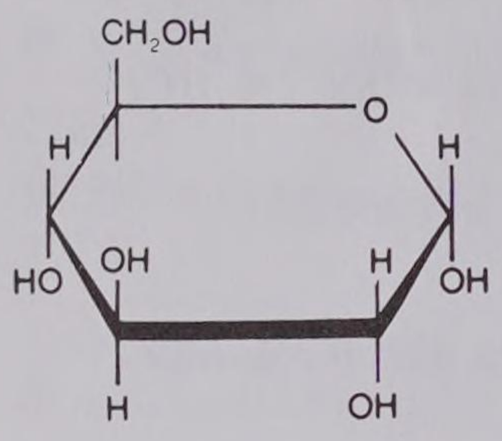


Figure 2.4 Structure of maltose, a disaccharide composed of 2 glucose units joined together.

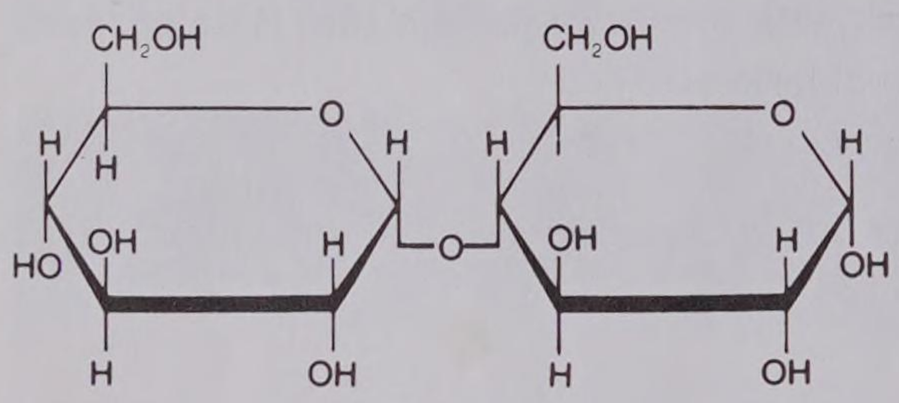
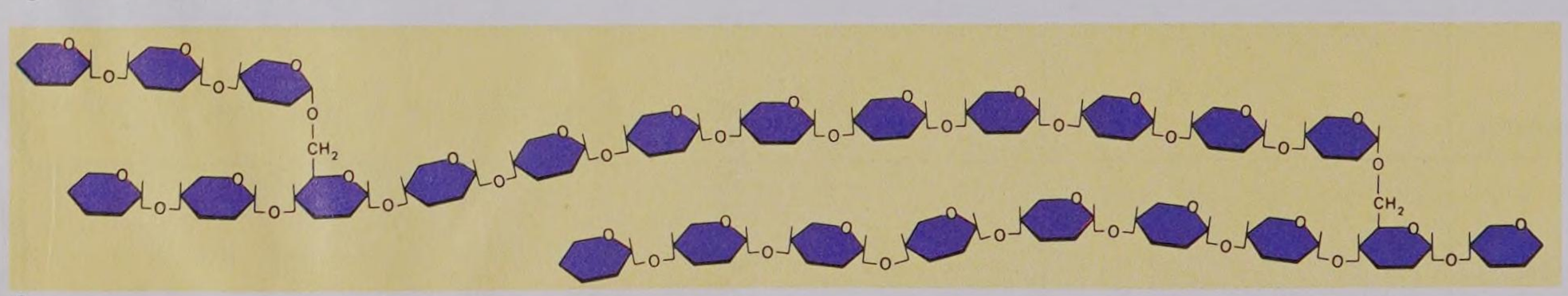
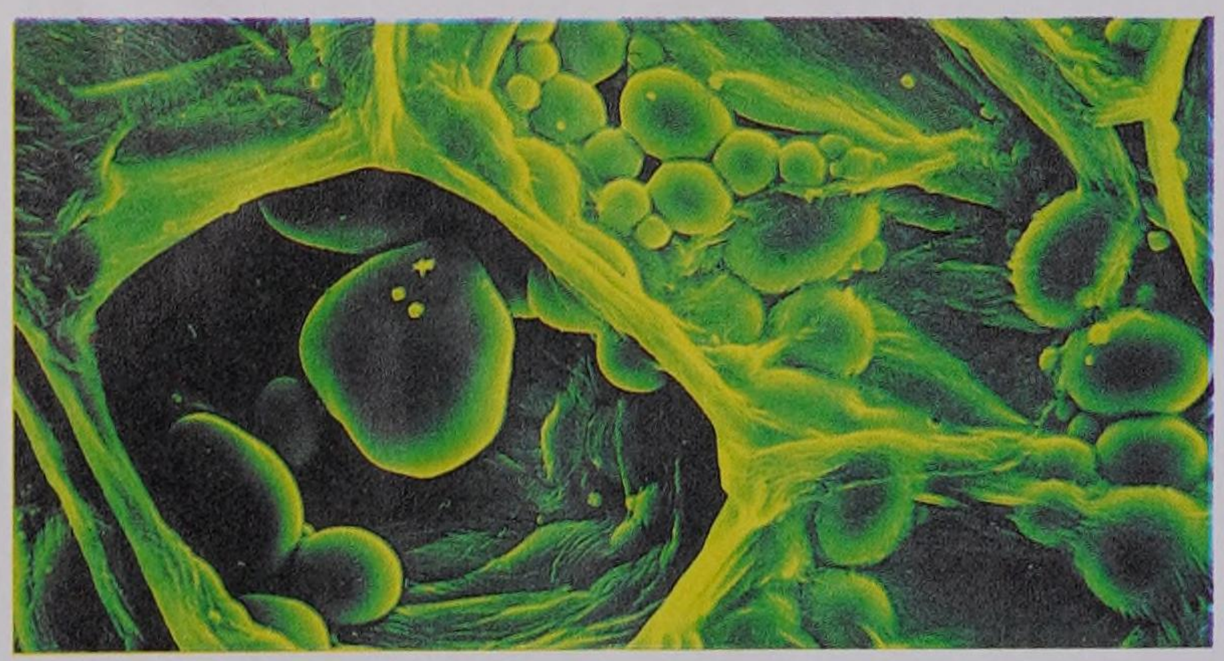


Figure 2.5 ***a.*** Starch is a polysaccharide composed of many glucose units. ***b.*** Scanning electron micrograph of starch granules in a plant cell.



a.



b.

Test for Starch

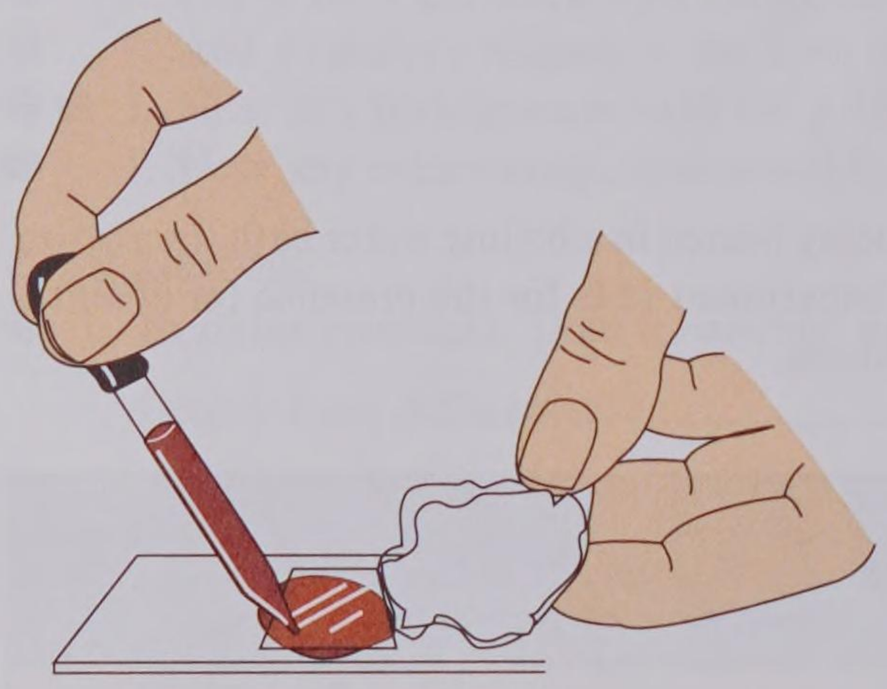
In the presence of (plant) starch, iodine solution (IKI) turns from a transparent orange-brown color to an opaque blue-black. Examine, as directed following, a thin slice of potato and onion to aid you in drawing conclusions about their starch composition.

Experimental Procedure

Experiment 1: Potato

S 1. With a sharp razor blade, slice a very thin piece of potato. Place it on a microscope slide, add a drop of *water* and a coverslip, and observe on low power with your compound light microscope. Compare your slide to the scanning electron micrograph of starch granules (fig. 2.5*b*). Find the cell wall (large geometric compartments) and the starch grains (numerous clear, oval-shaped objects). Without removing the coverslip, place 2 drops of *iodine* solution on the microscope slide so that it touches the coverslip. Draw the iodine under the coverslip by placing a small piece of paper towel in contact with the water on the opposite side of the coverslip (see fig. 2.6).

Figure 2.6 Application of iodine without removal of the coverslip.



S 2. Microscopically examine the potato again on the side closest to the point of application of the iodine solution.

What is the color of the small oval bodies? _____

What is the chemical composition of these oval bodies? _____

S Place the data from this experiment in table 2.4.

Experiment 2: Onion

S 1. With a razor blade, slice a small piece of onion and place it on a microscope slide.
S 2. Add a large drop of *iodine* solution.
S 3. Note the color.
S 4. Record in table 2.4.

S Additional test for starch: With a wax pencil, label 2 test tubes 3 and 4, and mark them both at the 1 cm level and treat as follows:

Experiment (Tube) 3

S 1. Fill to the 1 cm mark with *starch* solution (1%) and add 5 drops of *iodine* solution.
S 2. Note the final color, and record your results in table 2.4.

Experiment (Tube) 4

S 1. Fill to the 1 cm mark with *water* and add 5 drops of *iodine* solution. Note the final color, and record your results in table 2.4.

S 2. Compare your results with those around you. Note any differences here. _____
S 3. Use these results to draw conclusions about what organic compound is present (or absent) in each tube. Write these conclusions in table 2.4.

Table 2.4 Starch Test (Iodine Solution)

Exp. No.	Material	Observations	Conclusions
1	Potato	blue-black	Starch
2	Onion	no change	No
3	Starch solution	blue black	Starch
4	Water	no change	No

Starch Composition and Function

Sugars serve as an immediate energy source in cells. In plant cells, glucose (a primary energy molecule) is often stored in the form of starch.

Is glucose stored as starch in potato? yes

Is glucose stored as starch in onion? No

Test for Sugars

Specific sugars called *reducing sugars* react with *Benedict's reagent* after being heated in a boiling water bath. Increasing concentrations give a continuum of colored products (see table 2.5). This experiment tests for the presence (or absence) of varying amounts of reducing sugars in a variety of materials and chemicals.

Table 2.5 Benedict's Reagent (Some Typical Reactions)

Chemical	Chemical Category	Benedict's Reagent (after heating)
Water	Inorganic	Blue (no change)
Starch	Polysaccharide (carbohydrate)	Blue (no change)
Maltose	Disaccharide (carbohydrate)	Varies with concentration very low—green low—yellow moderate—yellow-orange high—orange very high—orange-red
Glucose	Monosaccharide (carbohydrate)	Varies with concentration—see maltose above

Experimental Procedure

Ⓢ Label and mark 5 test tubes at 1 cm and 3 cm.

Tube 1

Ⓢ 1. Fill to the 1 cm mark with *water*.
Ⓢ 2. Add *Benedict's* reagent to the 3 cm mark.
Ⓢ Ⓣ 3. Heat in a boiling water bath for 5–10 minutes.
Ⓢ 4. Note any color change, and record in table 2.6.

Tube 2

Ⓢ 1. Fill to the 1 cm mark with *glucose* solution.
Ⓢ 2. Add *Benedict's* reagent to the 3 cm mark.
Ⓢ Ⓣ 3. Heat in a boiling water bath for 5–10 minutes.
Ⓢ 4. Note any color change, and record in table 2.6.

Tube 3

1. Place a small amount of *onion juice* in a test tube. (Obtain the juice by macerating a small piece of onion with a mortar and pestle.)
2. Add *water* up to the 1 cm mark.
3. Add *Benedict's* reagent to the 3 cm mark.
4. Heat in a boiling water bath for 5–10 minutes.
5. Note any color change, and record in table 2.6.

Tube 4

1. Place a small amount of *potato juice* in a test tube. (Obtain the juice by macerating a small piece of potato with a mortar and pestle.)
2. Fill to the 1 cm mark with *water.*
3. Add *Benedict's* reagent to the 3 cm mark.
4. Heat in a boiling water bath for 5–10 minutes.
5. Note any color change, and record in table 2.6.

Tube 5

1. Fill to the 1 cm mark with *starch* solution.
2. Add *Benedict's* reagent to the 3 cm mark.
3. Heat in a boiling water bath for 5–10 minutes.
4. Note any color change, and record in table 2.6.

Conclusions

1. Examine your data. Does it basically agree with those around you? _____

   Describe any differences. _____

   _____

   _____

2. Then draw conclusions that indicate the kind and relative amount of chemical present in each tube, and enter in table 2.6.

Table 2.6 Benedict's Test

Tube No.	Content	Color (after heating)	Conclusions
1	Water	blue	no sugar
2	Glucose solution	orange	high concentration
3	Onion juice	yellow - orange	moderate
4	Potato juice	yellow	low
5	Starch solution	blue - may be yellow	No sugar

Starch Structure

The starch molecule is a polysaccharide (multiple sugar) comprised of 100s of glucose units (see fig. 2.5*a*). The action of the enzyme (protein) amylase breaks this large molecule into many disaccharide (double sugar) molecules of maltose.

$$\text{starch} + \text{water} \xrightarrow[\text{(enzyme)}]{\text{amylase}} \text{maltose}$$

Experimental Procedure

S 1. Mark 4 clean test tubes at 2 cm, 4 cm, and 6 cm levels and label these 1, 2, 4, and 5.
S 2. Mark 2 more tubes in the same way and at 8 cm. Label these 3 and 6.
S 3. Place these in numerical order in your test tube rack.
S 4. Fill and treat as directed.

Tube 1

S 1. Fill to the 2 cm mark with *water* and to the 4 cm mark with *amylase* (enzyme) solution.
S T 2. Shake and immediately test for sugar by adding *Benedict's* reagent to the 6 cm mark and heating in a boiling water bath for 5–10 minutes.
S 3. Record the color (after heating) in table 2.7.

Tube 2

BROWN

S 1. Fill to the 2 cm mark with *starch* and to the 4 cm mark with *amylase* (enzyme) solution.
S T 2. Shake and immediately test for sugar by adding *Benedict's* reagent to the 6 cm mark and heating in a boiling water bath for 5–10 minutes.
S 3. Record the color (after heating) in table 2.7.

NR 9

Tube 3

S 1. Fill to the 2 cm mark with *starch,* to the 4 cm mark with *pepsin* solution, and to the 6 cm mark with *hydrochloric acid* (0.2% HCl). (Pepsin is an enzyme found in the acid environment of the stomach.)
S T 2. Shake and immediately test for sugar by adding *Benedict's* reagent to the 8 cm mark and heating in a boiling water bath for 5–10 minutes.
S 3. Record the color (after heating) in table 2.7.

Tube 4

S 1. Fill to the 2 cm mark with *water* and to the 4 cm mark with *amylase* (enzyme) solution.
S T 2. Shake and wait for 30 minutes. At the end of this time, test for sugar by adding *Benedict's* reagent to the 6 cm mark and heating in a boiling water bath for 5–10 minutes.
S 3. Record the color (after heating) in table 2.7.

Tube 5

S 1. Fill to the 2 cm mark with *starch* and to the 4 cm mark with *amylase* (enzyme) solution.
S T 2. Shake and wait for 30 minutes. At the end of this time, test for sugar by adding *Benedict's* reagent to the 6 cm mark and heating in a boiling water bath for 5–10 minutes.
S 3. Record the color (after heating) in table 2.7.

Tube 6

S 1. Fill to the 2 cm mark with *starch,* to the 4 cm mark with *pepsin* solution, and to the 6 cm mark with *hydrochloric acid* (0.2% HCl). (Pepsin is an enzyme found in the acid environment of the stomach.)
S T 2. Shake and wait for 30 minutes. At the end of this time, test for sugar by adding *Benedict's* reagent to the 8 cm mark and heating in a boiling water bath for 5–10 minutes.
S 3. Record the color (after heating) in table 2.7.

Table 2.7 Starch Composition and Enzyme Specificity

Tube No.	Content	Color (final)	Conclusions
1	Water Amylase (immediate)	blue	NO sugar
2	Starch Amylase (immediate)	blue/yellow	May be Some sugar

Table 2.7 Continued

Tube No.	Content	Color (final)	Conclusions
3	Starch Pepsin HCl (immediate)	Same	as #2
4	Water Amylase (30 min)	blue	No sugar
5	Starch Amylase (30 min)	Orange	high concentration of sugar
6	Starch Pepsin HCl (30 min)	Same as #2	

Conclusion

1. Evaluate the data entered in table 2.7. Compare your data with others around you. Does it agree? _____

   If there are discrepancies, state them here. _____

   _____

2. Consider the data obtained (by you and others around you), the basic nature of each compound, and the specificity of enzymes for given substrates and write a conclusion for each tube that includes both the final products present and what occurred (or did not occur) for these products to be present.
3. Recall that earlier you predicted that pepsin would have no effect on starch. Is that statement supported by these results? Explain.

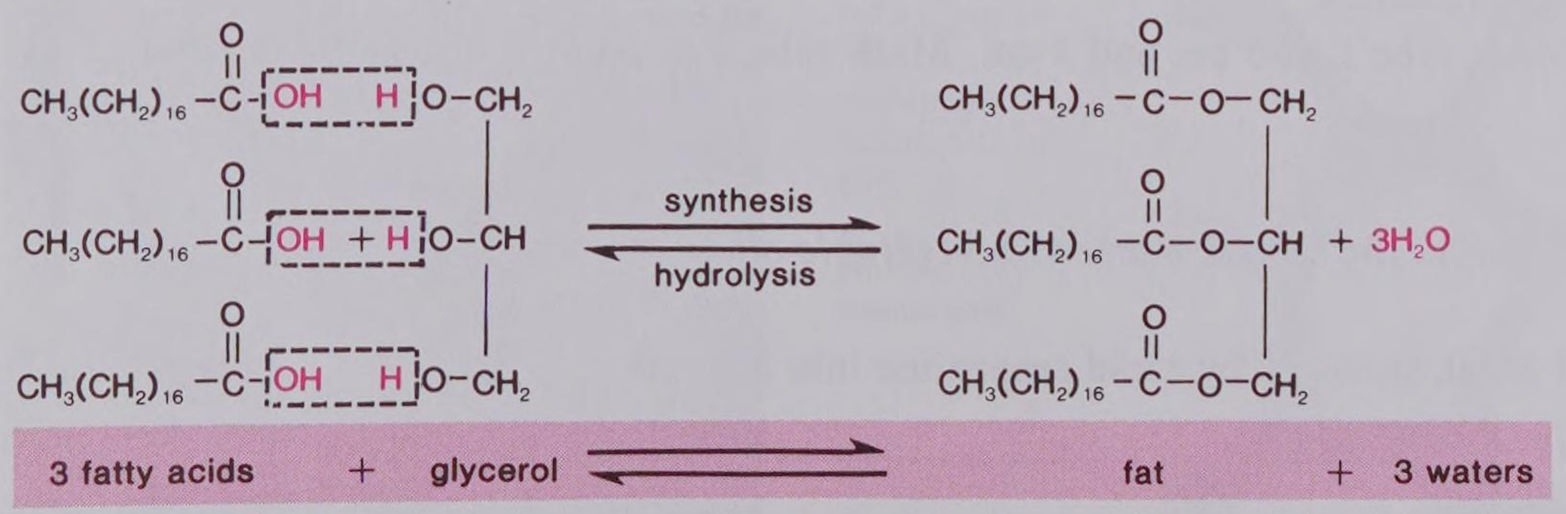
   _____

   _____

Lipids

Lipids are compounds that are insoluble in water and soluble in solvents such as alcohol and ether. Lipids include fats, oils, phospholipids, steroids, and cholesterol. Typically, fats and oils are composed of one molecule of glycerol bonded to 3 molecules of fatty acids (fig. 2.7).

Figure 2.7 When glycerol joins with 3 fatty acids, a neutral fat molecule results.



Test for Lipids

Experimental Procedure

S 1. Place a small drop of *water* on a square of brown paper. Describe the immediate effect. _____

_____

S 2. Place a small drop of *vegetable oil* on a square of brown paper. Describe the immediate effect. _____

_____

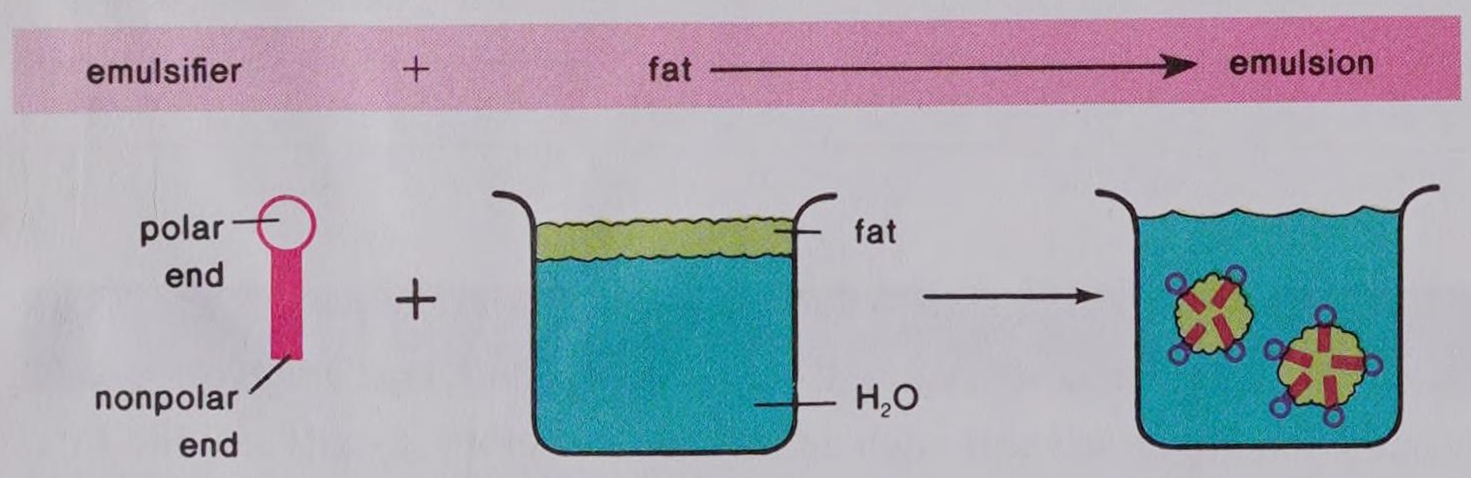
T S 3. Wait at least 5 minutes. Evaluate which substance impregnates the paper and which is subject to evaporation. Record your results in table 2.8.

Table 2.8 Test for Lipids

Sample	Results
Water spot	
Oil spot	

Emulsifying compounds, because of their structural nature (see fig. 2.8) surround small droplets of oil and allow them to be dispersed. Without emulsifiers, mixing would cause only temporary dispersion of the oil and it would quickly float on the top of the water because of its lower density. Emulsifiers, such as lecithin from egg yolk, are used to keep the oil in salad dressing dispersed. Other emulsifiers include bile salts, produced by the liver and released into the small intestine, and Tween, a commercial emulsifier.

Figure 2.8 An emulsifier contains molecules with polar and nonpolar ends. When the nonpolar ends are attached to the nonpolar fat, the polar ends are exposed. Since the polar ends are soluble in water, the fat becomes dispersed.



Emulsification of Lipids

Experimental Procedure

This experiment illustrates the effect of emulsifiers.

S Label 2 test tubes 1 and 2. Mark tube 1 at 3 cm and 4 cm. Mark tube 2 at 2 cm, 3 cm, and 4 cm and fill as follows:

Tube 1

S 1. Fill to the first mark with *water* and to the second mark with *vegetable oil.*

S 2. Shake.

S 3. Observe for the initial dispersal of oil, followed by rapid separation into 2 layers.

Is vegetable oil soluble in water? _____

Tube 2

S 1. Fill to the first mark with *water,* to the second mark with *vegetable oil,* and to the third mark with the available emulsifier (*Tween* or *bile salts*).

S 2. Shake.

3. Describe how the distribution of oil in this tube compares with the distribution in tube 1. _____

_____

Microscopic Examination

T S 1. Let both tubes settle for 5 minutes. Label 2 microscope slides 1 and 2.

S 2. Using different droppers, remove a sample of the solution that is just below the layer of oil, add a coverslip, and examine with the low power of your compound light microscope.

S 3. Record your observations in table 2.9.

Table 2.9 Emulsification Observations (Microscopic)

Tube No.	Content	Observations
1	Oil Water	
2	Oil Water Emulsifier	

4. Explain the correlation between your macroscopic observations (how the tubes look to your unaided eye) and your microscopic observations.

_____

_____

Fat in Cells

In animals, adipose (fat) tissue is found beneath the skin and around the various internal organs. These cells contain a droplet of fat, a molecule that provides for long-term energy storage. When more fat is added to this tissue, the droplet increases in size, not in the number of fat cells.

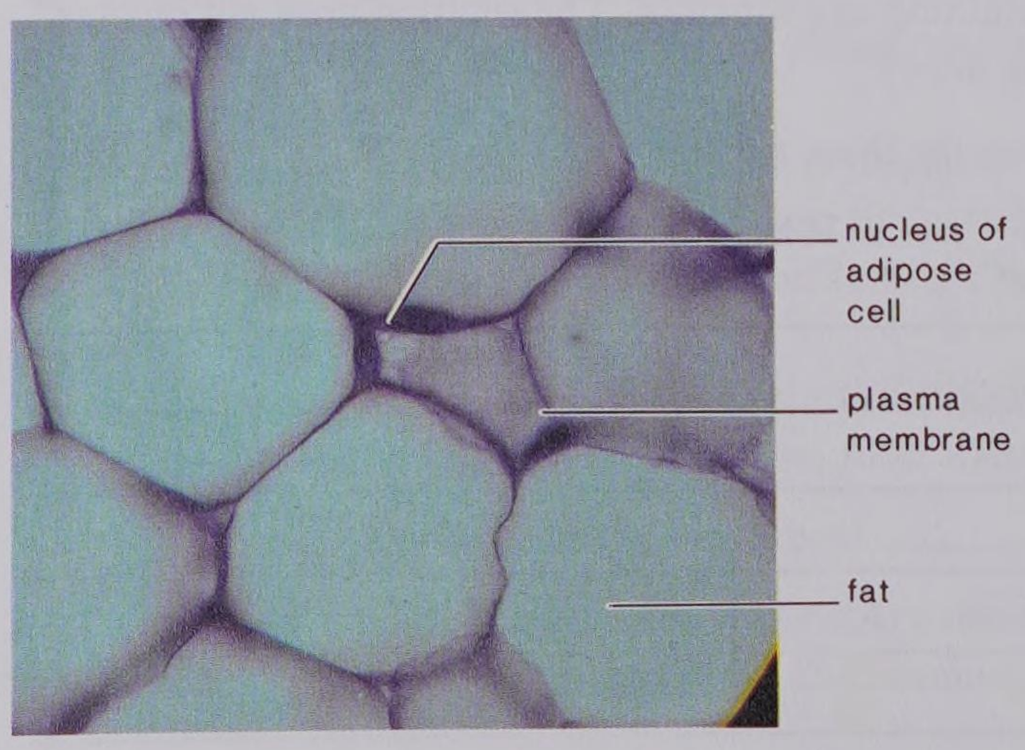
Experimental Procedure

S 1. Obtain a prepared microscope slide of adipose tissue, and view it under the light microscope.

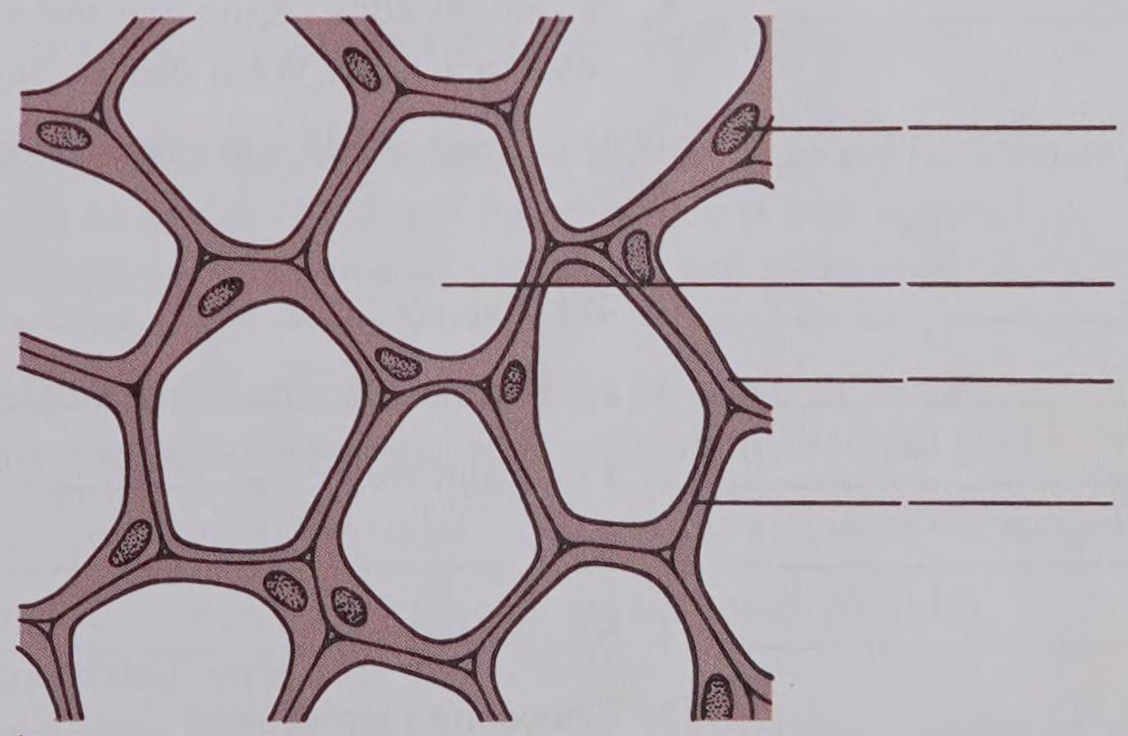
S 2. Compare it to figure 2.9 of adipose tissue.

S 3. Complete the labeling of figure 2.9*b*.

Figure 2.9 Adipose tissue. Notice how the fat droplets push the cytoplasm to the edges of the cells.



a.



b.

Testing Chemical Composition of Everyday Materials and an Unknown

There are several everyday materials provided for you. Using the tests for the carbohydrates (reducing sugars and starches), proteins, and lipids in this exercise, determine the macromolecules present in these materials.

1. Write the *name* of each known material and the *letter* of the unknown provided.
2. Record your results in table 2.10.

Table 2.10 Everyday Materials and Unknowns

Sample Name	Reducing Sugar (Benedict's)	Starch (iodine)	Protein (biuret)	Lipid (brown paper)
apple	yes			yes
potato		yes		
onion				
banana	yes		yes	yes
gum	yes			
Ritz Bits (peanut butter + cracker)				yes
				yes
Unknown				
Unknown				

Laboratory Review 2

1. What metallic ion does the amino group complex with in the biuret test?
2. What purpose does a water sample serve when you establish a test for a particular compound, such as in table 2.2?
3. If you add iodine or IKI solution and it turns purple-black, what macromolecule is present?
4. You are required to test a solution for the presence of glucose. What test would you use?
5. Is it necessary to heat the Benedict's reagent in boiling water for it to react?
6. A student adds iodine to a test tube containing egg albumin. The solution does not change color in 1 hour. What conclusions can be drawn?

   What test would you expect to be positive for these substances? Why?

Test		Why?
	7. Table sugar	
	8. Peach juice	
	9. Flour and water	
	10. Milk	
	11. Salad dressing	
	12. Chocolate chip cookies	

13. You discovered that pepsin has no action in starch. What does this tell you about enzymes in general?

Laboratory

3

Cell Structure and Function

Learning Objectives

Students should be able to

1. identify the parts of animal and plant cells in the figures;
2. state the functions of the parts of animal and plant cells;
3. give examples of animal and plant tissues;
4. define and describe the process of diffusion as exemplified by the experimental procedure used here;
5. define isotonic, hypertonic, and hypotonic solutions, and give examples in terms of concentrations of NaCl;
6. predict the effects of these solutions on red blood (animal) cells and on *Elodea* (plant) cells.

Introduction

The cell theory states that the cell is the basic unit of life and that all living organisms are composed of one or more cells. Modern biologists recognize that there are 2 types of cells—prokaryotic and eukaryotic. *Prokaryotic cells* (true bacteria and blue-green bacteria), which lack a nuclear envelope and membranous cytoplasmic organelles (specialized structures within cells), are studied in a later laboratory. *Eukaryotic cells* possess organelles surrounded by membranes and a nucleus enclosed by a nuclear envelope. These are characteristics of complex cells.

In this laboratory you will study eukaryotic cells and the various organelles found in them. In addition, you will study diffusion and test the effects of solutions with various tonicities on red blood cells and *Elodea* cells.

Amoeba

An amoeba is a single-celled animal that shows the characteristics of a eukaryotic cell. You will study it using the compound light microscope, as early microscopists did.

S 1. Make a wet amount of *Amoeba* by using a drop of the culture (taken from the bottom of the container).

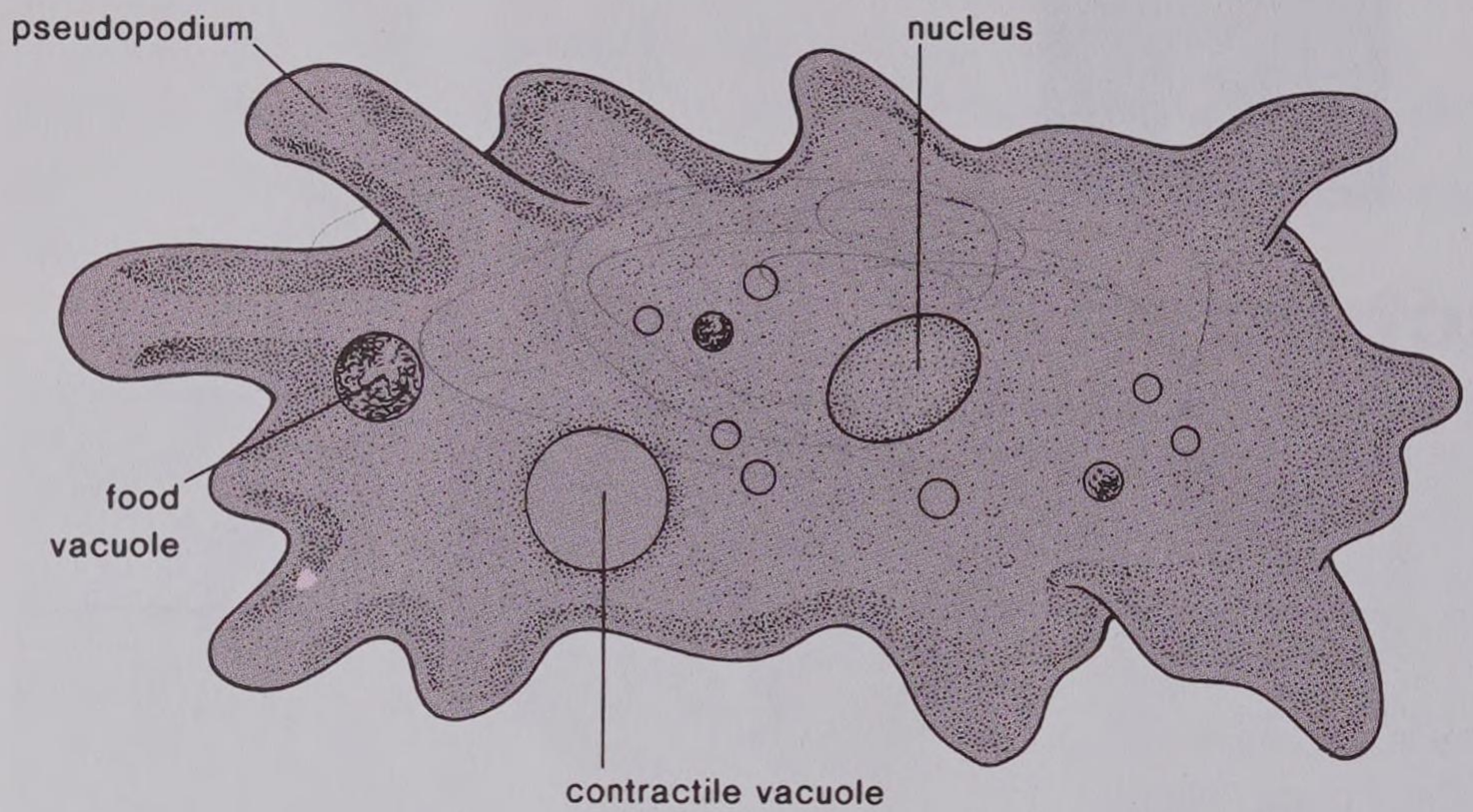
S 2. Observe the movement of this single-celled organism.

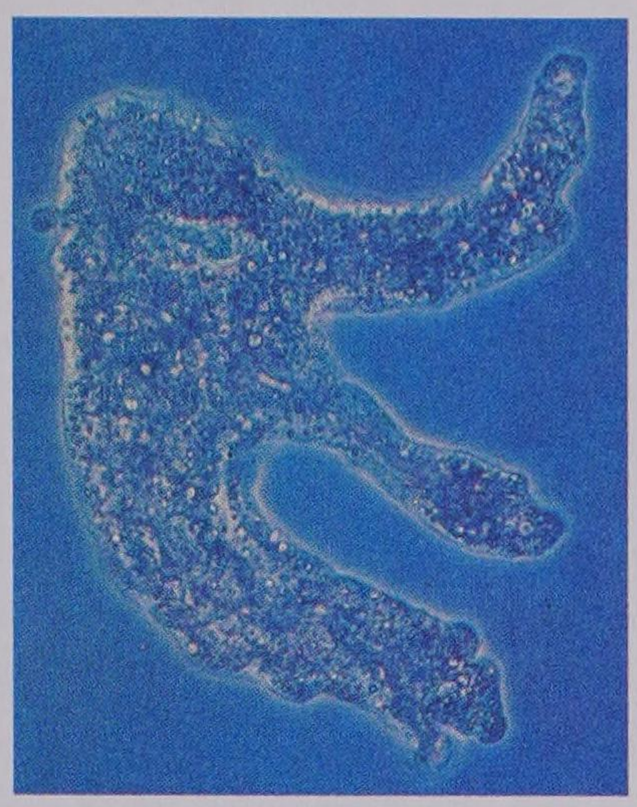
S 3. Study carefully the formation of *pseudopodia,* extensions of cytoplasm that give amoeba their irregular appearance.

S 4. Try to see the transition from *sol* (cytoplasm in solution), when the pseudopodia are forming, to *gel* (cytoplasm having a gelatin consistency), when the pseudopodia are no longer forming. This transition is now believed to be due to the contraction of microfilaments that are visible only with special techniques and high-powered microscopes.

S 5. You may be able to identify within the amoeba the following structures (see fig. 3.1): *nucleus*—a single membrane-bounded oval structure; *food vacuoles*—membrane-bounded spheres that contain engulfed food during digestion. (Are these present? _____); *contractile vacuoles*—transparent membrane-bounded spheres used to expel excess water. (Are these present? _____); the numerous small *granular structures* are various organelles that cannot be differentiated under this magnification.

Figure 3.1 ***a.*** Diagrammatic representation of an amoeba. ***b.*** Photomicrograph of *Amoeba proteus*.





Animal Cell Structure

The fine structure shown in the animal cell model (if available) and in figure 3.2, is based primarily upon electron microscope data.

1. First, note that the cell contains a transparent background substance called *cytosol.* The term *cytoplasm* refers to all the contents of the cell between the nucleus and plasma membrane. Various structures within the cytoplasm are organelles. The fluid portion of the nucleus is called *nucleoplasm.*
2. Identify the structures in the following list, observe their shape and location in figure 3.2, and copy a function for each from table 3.1.

Structure	**Function**
Plasma membrane	
Mitochondria	
Lysosomes	
Vacuoles	
Endoplasmic reticulum	
Rough endoplasmic reticulum	
Smooth endoplasmic reticulum	
Golgi apparatus	
Centrioles	
Nucleus	
Nucleolus	
Chromatin	

Figure 3.2 Diagrammatic representation of an animal cell.

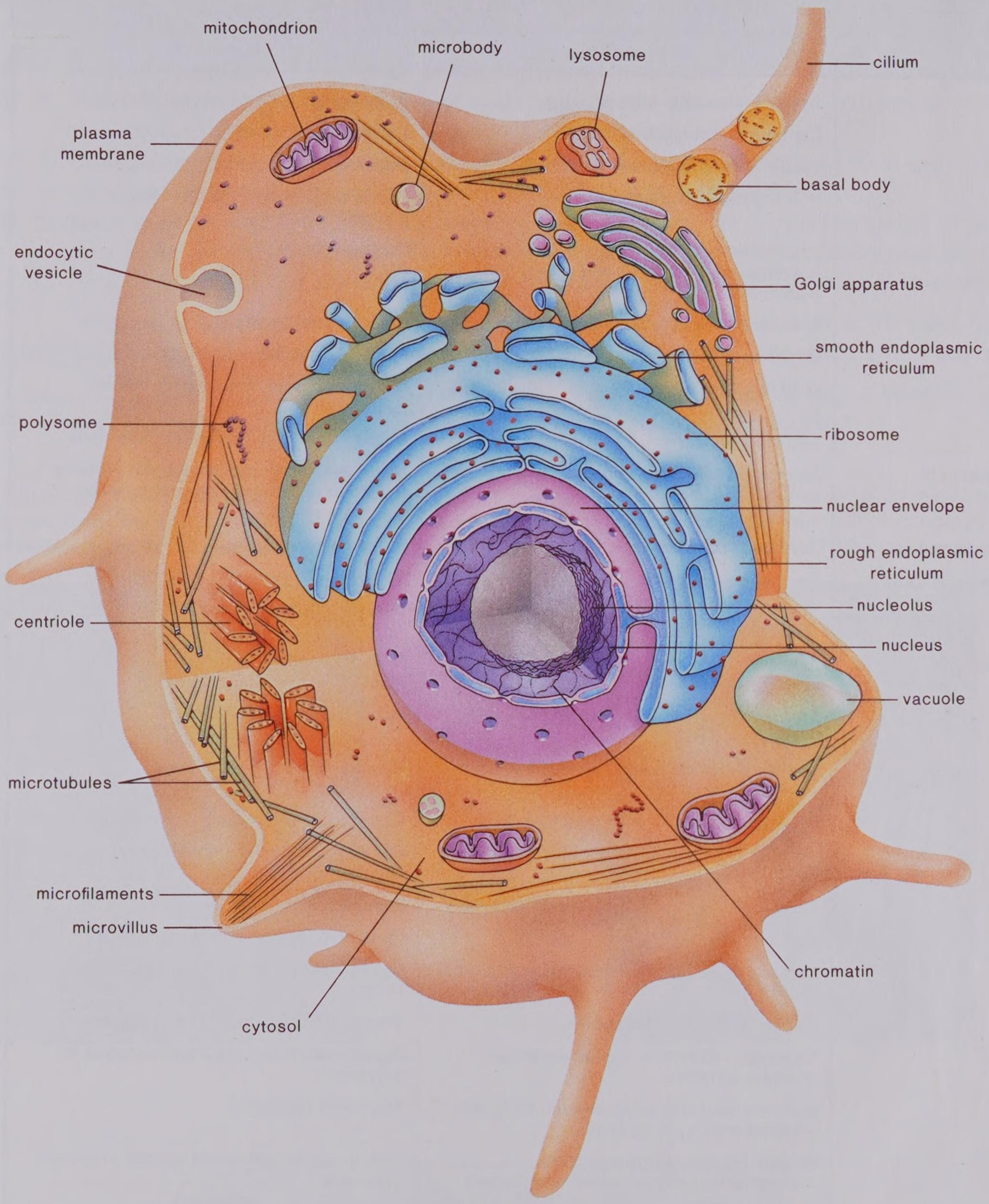


Table 3.1 Eukaryotic Structures in Animal and Plant Cells

Name	Composition	Function
Basal body	9 + 0 microtubule triplets	Organize structure of cilia and flagella
Cell wall*	Contains cellulose fibrils	Support and protection
Central vacuole	Water, ions, sugars, some proteins	Water and ion reservoir
Centriole†	9 + 0 pattern of microtubules	Forms basal bodies that give rise to microtubules
Chloroplast*	Inner membrane (grana) within 2 outer membranes, chlorophyll and accessory pigments	Photosynthesis
Chromatin	DNA strands and associated proteins	Hereditary information
Cilia† and flagella	9 + 2 pattern of microtubules	Movement of cell
Cytosol	Fluid medium of water, amino acids, ions, etc.	Reservoir of raw materials for cellular synthesis
Cytoskeleton	Microtubules and microfilaments	Shape of cell and movement of its parts
Endoplasmic reticulum (ER)	Membranous flattened channels and tubular canals	Synthesis and/or modification of proteins and other substances and transport by vesicle formation
Endoplasmic reticulum, rough	Studded with ribosomes	Protein synthesis
Endoplasmic reticulum, smooth	Having no ribosomes	Various; lipid synthesis in some cells
Endocytic vesicle	Membranous sac containing engulfed material (i.e., bacteria proteins)	Digest contents when fused with lysosomes
Golgi apparatus† (dictyosome)*	Stack of membranous sacs	Processing and packaging of macromolecules, e.g., glycoproteins
Lysosome†	Membranous vesicle containing digestive enzymes	Intracellular digestion
Microbodies	Membranous vesicle containing specific enzymes	Various metabolic tasks
Microfilaments	Thin fibers (7.5 nm) of actin (protein)	Constriction of microvilli, plasma membrane
Microtubules	Small (30 nm) cylinders of tublin (protein)	Cell shape, organelle movement, cilia, and flagella movement
Microvillus†	Cell projections containing microfilaments	Increases cell surface area
Middle lamella*	Pectin (polysaccharide)	Cementing agent between cell walls
Mitochondrion	Folded inner membrane (cristae) within outer membrane	Cellular respiration—energy production (ATP)
Nuclear envelope	Double membrane with pores	Separation of nucleus from cytoplasm
Nuclear pore	Opening (100 nm) in nuclear envelope, complex structure	Passage of ribosomes from nucleolus to cytoplasm
Nucleolus	Concentrated area of chromatin, RNA, and proteins within the nucleus	Ribosome formation
Nucleus	Nuclear envelop surrounding the nucleoplasm, chromosomes, and nucleoli	Cellular reproduction and control of protein synthesis
Plasma membrane	Bilayer of phospholipid with embedded proteins	Selective passage of molecules into and out of cell, cell recognition and location of some enzymes
Polysome (polyribosome)	A group of ribosomes	Involved in producing copies of the same protein
Ribosomes	Protein and RNA in 2 subunits	Protein synthesis
Starch grains*	Starch (polysaccharide of glucose units)	Energy storage
Vacuoles and vesicles	Membranous sacs	Storage of substances

*Plant cells only

†Animal cells only

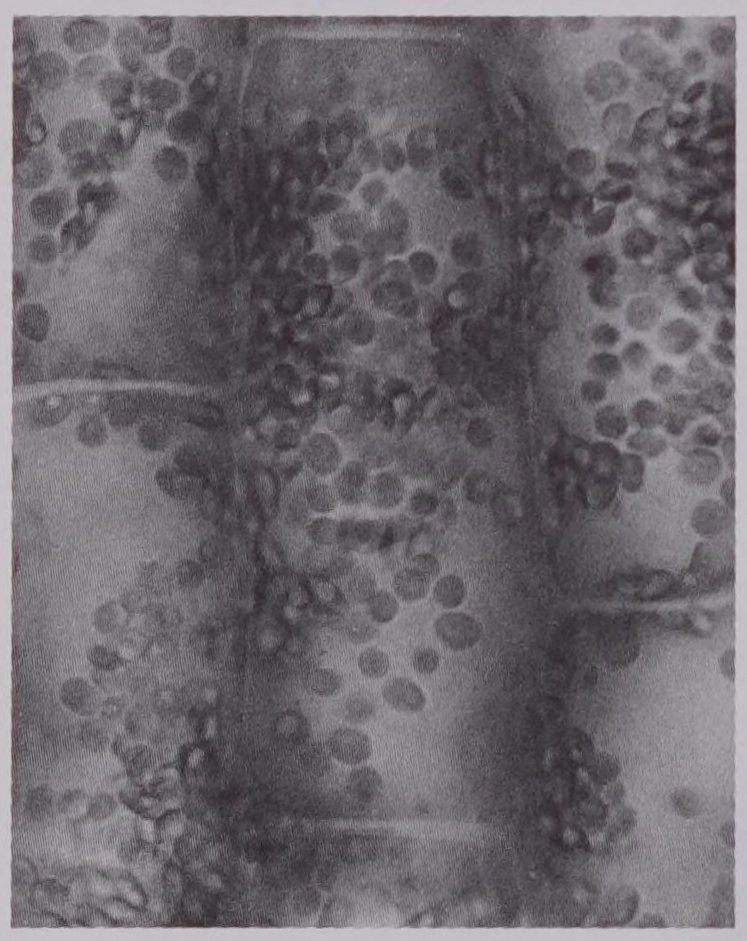
Elodea (Anacharis)

Elodea is a multicellular eukaryotic plant found in freshwater ponds and lakes.

1. Prepare a wet mount of a small piece of *Elodea* leaf in fresh *water.*
2. Select a young leaf and have the drop of *water* ready on your slide so that the leaf does not dry out, even for a few seconds. Take care that the leaf is mounted with its top side up.
3. Examine the slide using low power, making sure to focus sharply on the surface of the leaf.
4. Select a cell with numerous chloroplasts for further study and switch to high power.
5. Focus carefully until the side and end walls are exactly in focus. The chloroplasts appear to be only along the sides of the cell because the large, fluid-filled membrane-bounded, central vacuole pushes the cytoplasm against the cell walls (fig. 3.3*a*). Then focus on the surface and notice an even distribution of chloroplast.

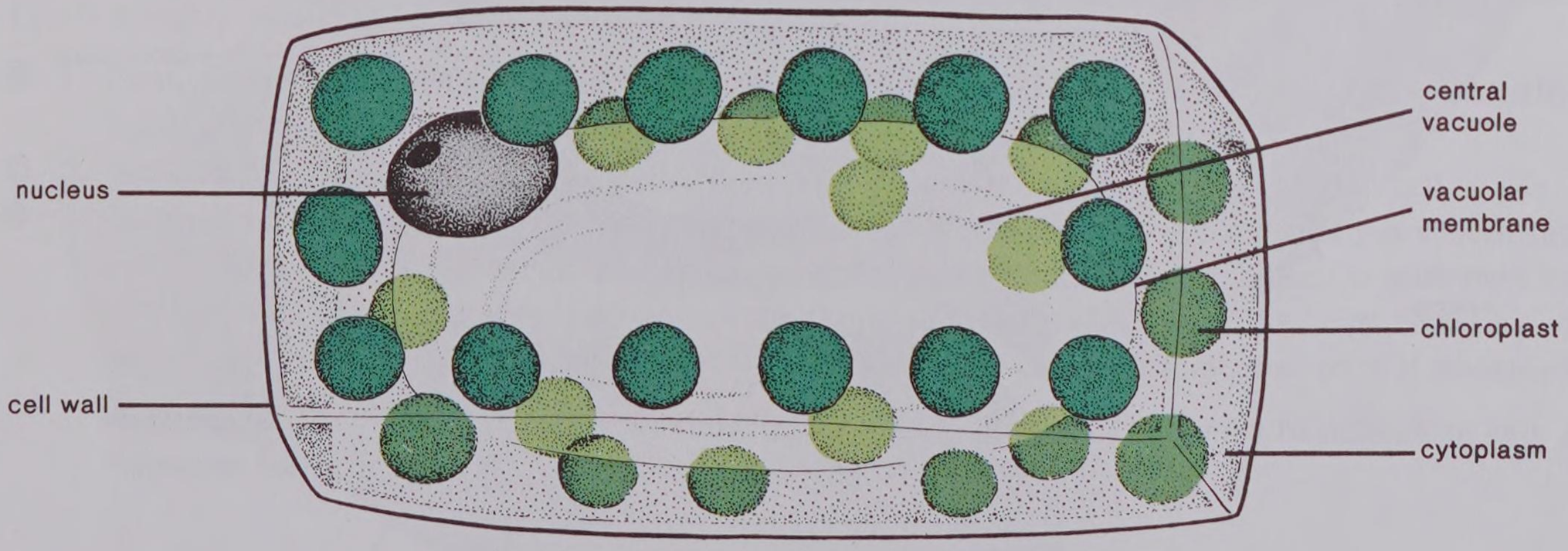
Can you *locate* a nucleus? _____ It may be hidden by the chloroplast, but when visible it appears as a faint gray lump on one side of the cell. Can you detect movement of the chloroplast in this cell or any other cell? _____The chloroplasts are not moving under their own power but are being carried by a streaming of the nearly invisible cytoplasm. Save your slide for use at the end of this laboratory.

Figure 3.3 *Elodea* cells. ***a.*** Upper surface of cells showing an even distribution of chloroplast (oval structures). ***b.*** Middle of the cell with chloroplast visible around the perimeter and not in the center, which is occupied by a membrane-bound, fluid-filled central vacuole. ***c.*** A diagrammatic representation of a single *Elodea* cell.



a.

b.



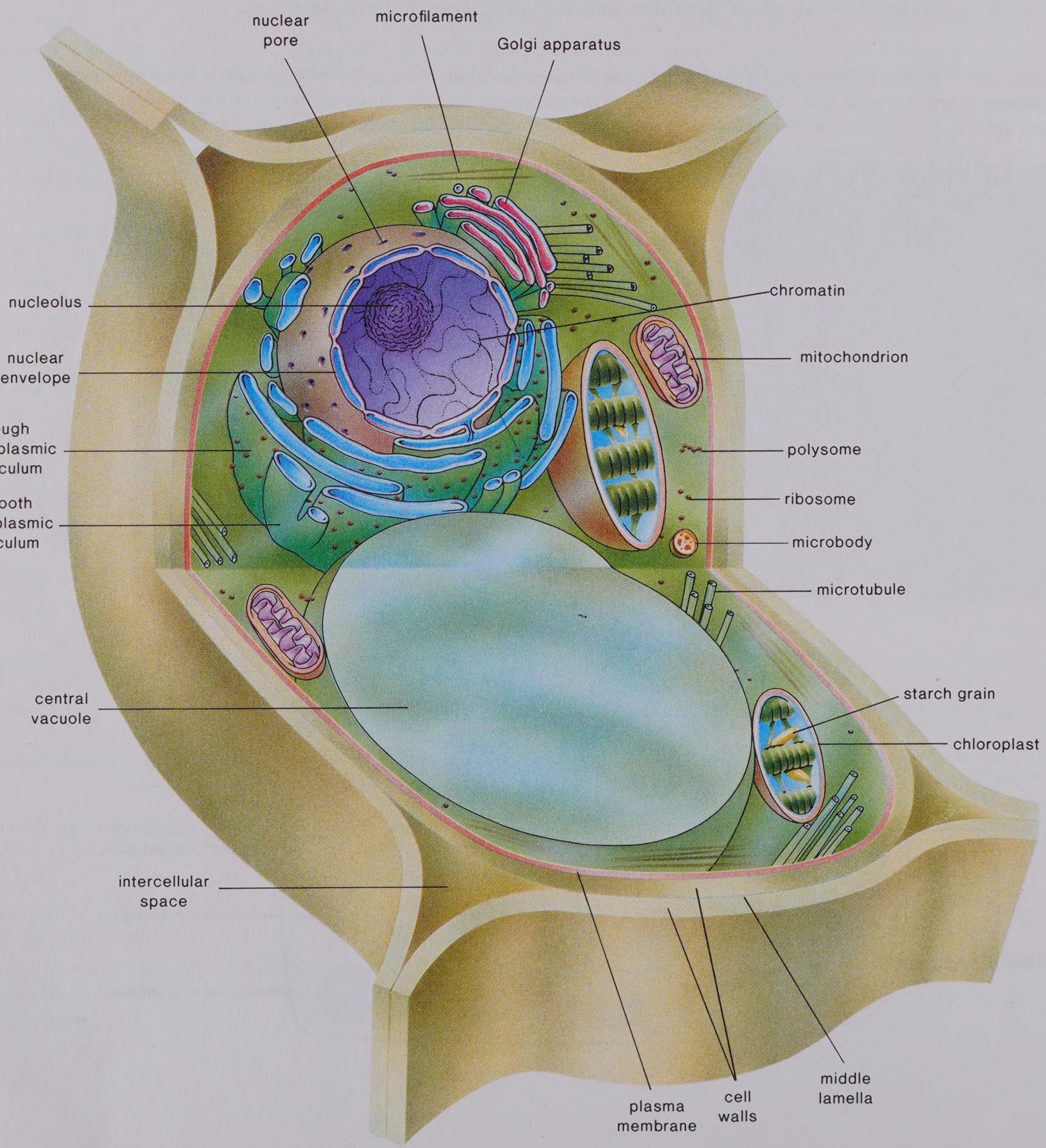
c.

Plant Cell Structure

Plant cells contain all of the structures mentioned as being present in animal cells except lysosomes, centrioles, and microvilli. Compare the similarity of eukaryotic animal cell (fig. 3.2) and eucaryotic plant cell (fig. 3.4) structures. In addition, identify the following plant structures using table 3.1 and figure 3.4 and copy a function:

Structure	Function
Cell wall	_____
Central vacuole, large	_____
Chloroplasts	_____
Middle lamella	_____
Starch grains	_____

Figure 3.4 Diagrammatic representation of a plant cell.



Differentiated Cells

In multicellular organisms, most cells have a specific structure and function. Usually, cells with similar structure and function make up a *tissue*.

Animal Tissue Examples

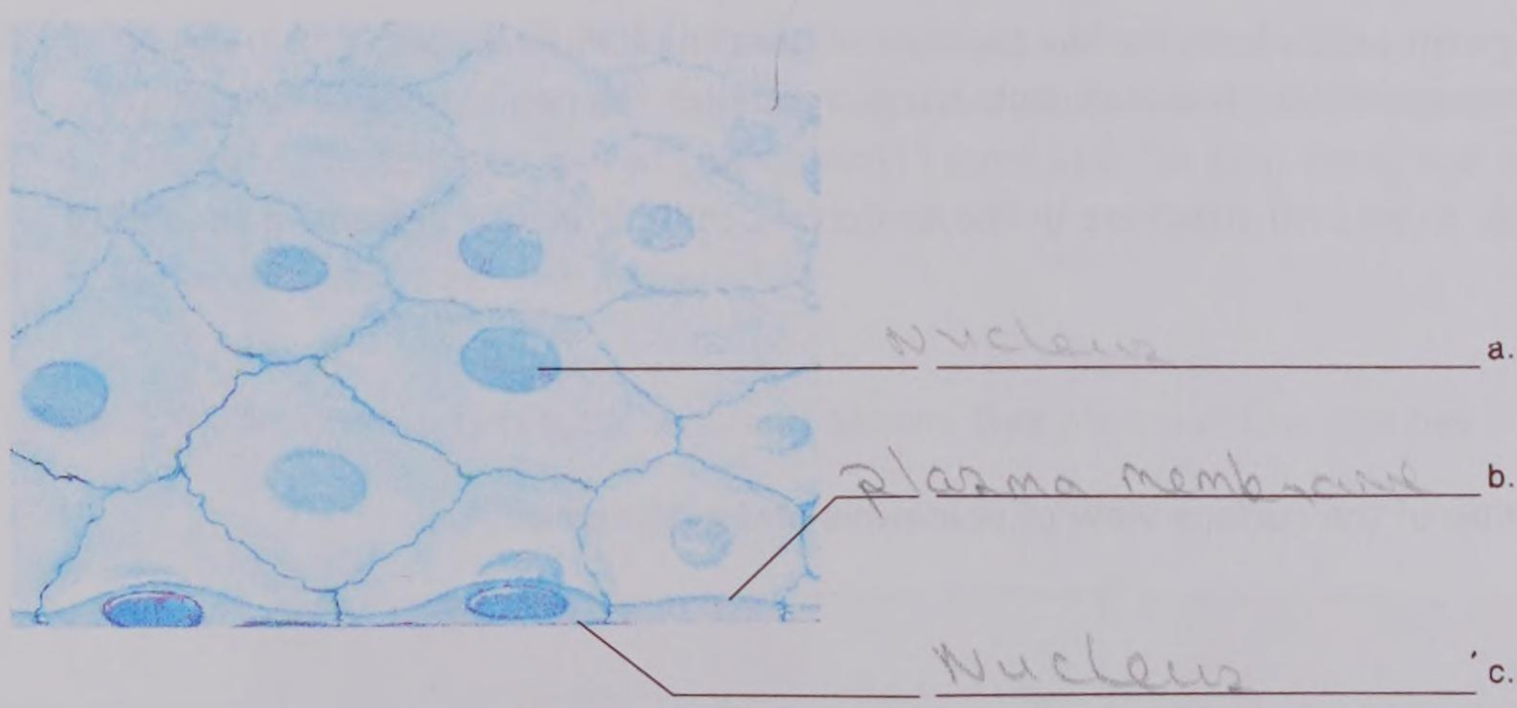
In vertebrates, epithelial tissue forms a continuous layer or sheet over the entire body surface and most of the body's inner cavities. It serves to protect. On internal surfaces, this tissue may be specialized for other functions such as protection and absorption.

Squamous Epithelium

Squamous epithelium occurs as a sheet of flat, platelike (squamous) cells. This is the least specialized of the epithelial tissues.

1. Study figure 3.5 of squamous epithelium and then examine the prepared slide of a surface view of frog skin.
2. In low power, note the close packing of the flat cells. What is the shape of the cells? _____
3. In high power, examine an individual cell and identify the *plasma membrane, cytoplasm,* and *nucleus*. What is the diameter (p. 9) of your high power field (μm)? (Recall its computation from Laboratory 1.) 500
4. Estimate the size of an epidermal cell (μm). _____
5. Recall your observation of human cheek cells (p. 11). What type of tissue lines the mouth? Squamous epithelial
   The function of this tissue is protection.

Figure 3.5 Simple squamous epithelial tissue. (Courtesy Kendall/Hunt Publishing Company. © 1975 Kendall/Hunt Publishing Co.)

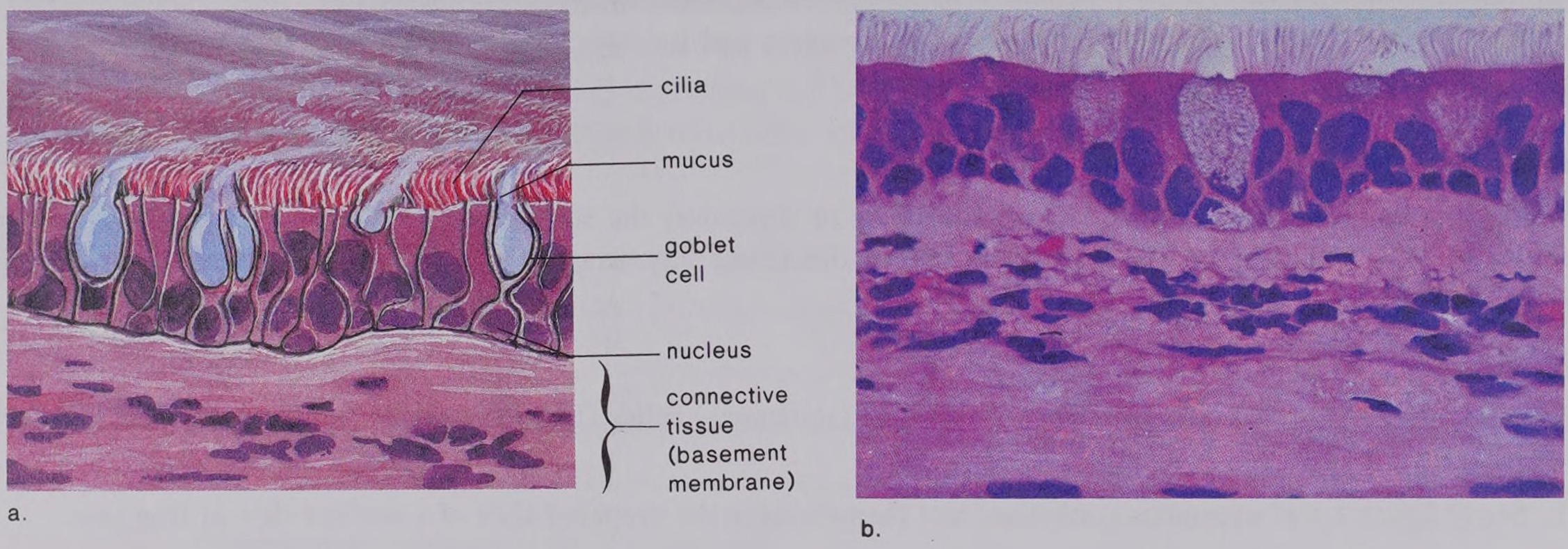


Pseudostratified Ciliated Columnar Epithelium

This is a highly specialized form of epithelial tissue.

1. First, study figure 3.6. It is called *pseudostratified* because while it appears to be layered, actually all cells touch the underlying connective tissue (basement membrane).
2. Note the fine hairlike projections, called cilia, that are located on the free end of each cell.
3. Now examine the prepared slide of a section of human trachea (windpipe). Be sure you concentrate on that part of the slide that has the general appearance of the diagram. In the windpipe, the cilia wave back and forth, moving mucus and debris up toward the throat so that it cannot enter the lungs. Smoking and prolonged breathing of air pollutants, have been shown to destroy these cilia, but they grow back if smoking is discontinued. Are cilia visible on your slide? _____ These may be difficult to find; ask your instructor for help.

Figure 3.6 Pseudostratified ciliated columnar epithelial tissue from trachea. ***a.*** Diagram. ***b.*** Photomicrograph.



Plant Tissue Example

Most cells in a plant's body, as well as in an animal's body, are specialized to perform a particular function. As an example, examine figure 3.7, the surface view of a flowering plant's leaf epidermis. The actual living epidermis may be observed in a future laboratory.

Identify and label the following structures and cells on figure 3.7:

Epidermal cells: These are the cells that look like jigsaw pieces. Like epithelial tissue in animals, epidermal tissue in plants protects the body from injury and drying out.

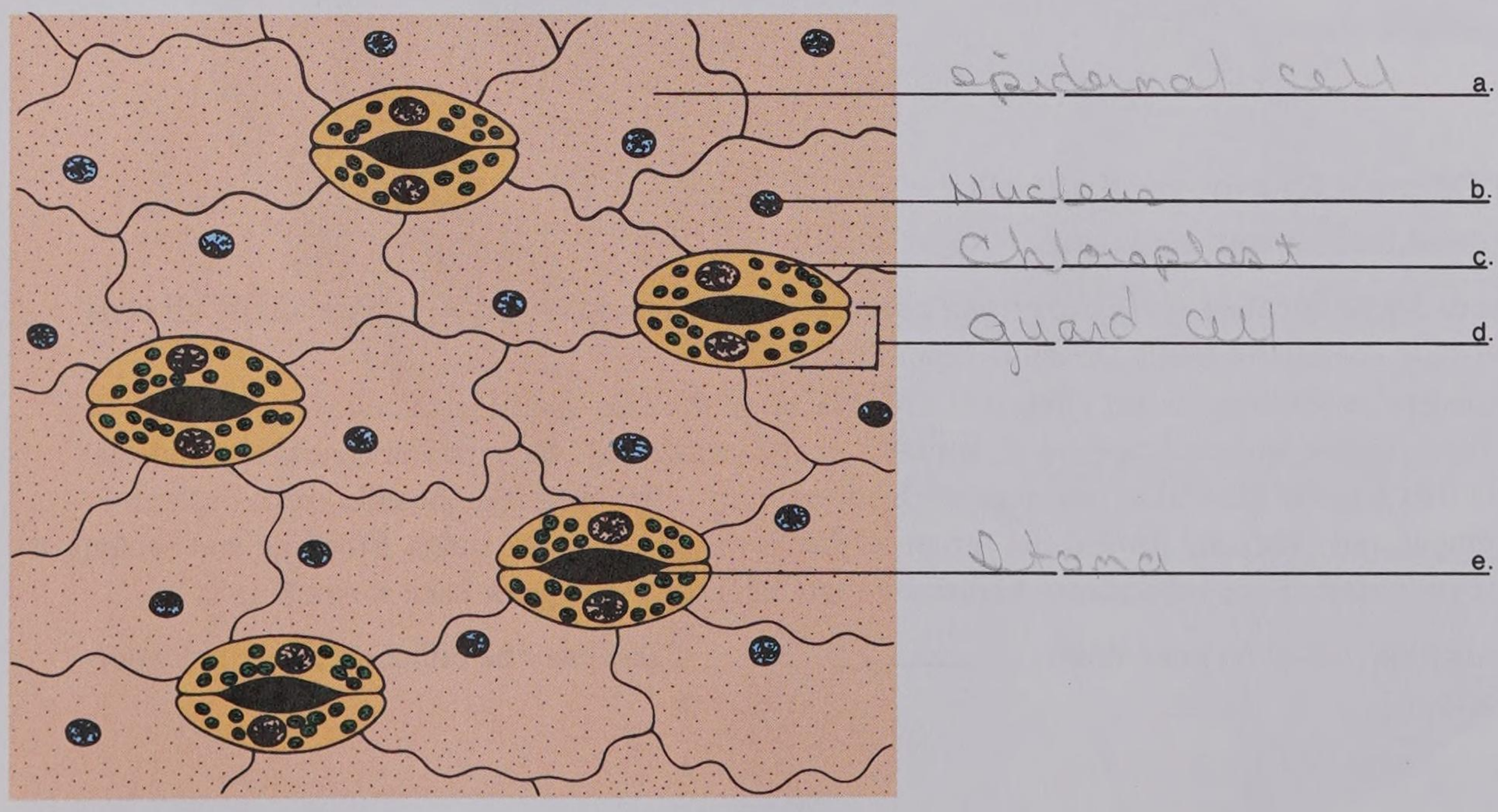
Guard cells: These are high specialized cells that regulate the opening and closing of the stomata (openings that permit air to enter the leaf).

Stoma (pl. stomata): The opening of the guard cell; allows for the passage of gases and water vapor.

Chloroplast: These chlorophyll (green photosynthetic pigment) containing organelles are present only in the guard cells of the leaf epidermis.

Nucleus: A single nucleus is usually visible as an oval structure in the epidermal cells. It is also present in the guard cells but is not readily visible.

Figure 3.7 A diagrammatic representation of the surface view of epidermis and guard cells.



Passive Transport Processes

The processes demonstrated in the remaining exercises are all considered passive transport processes because there is no cellular energy expended in the processes. The driving force behind these molecular movements is the natural kinetic energy of molecules. The kinetic energy of a substance is dependent upon its physical state (gas—highest; liquid—less; solid—least); its temperature (the higher the greater movement); and the molecular size (the smaller the faster).

Diffusion

Diffusion is the movement of molecules from an area of their *greater* concentration to an area of their *lesser* concentration. In order to demonstrate the process of diffusion, perform the following 3 diffusion experiments.

Experiment 1

S 1. Obtain a 1.5% gelatin (or agar) tube to which dye was added a few days ago and placed in a horizontal position (fig. 3.8).
S 2. Record the starting time from the instructor and complete the remaining measurements and calculations.
S 3. Enter the data in table 3.2.

Experiment 2

S 1. When the instructor sprays an aerosol spray in the corner of the room, note the time zero and the final time (for spray to reach you). Measure the movement (distance) (mm), and calculate the speed of diffusion (mm/hr).
S 2. Enter data in table 3.2 (fig. 3.9).

Experiment 3

S 1. Place very few crystals of *potassium permanganate* ($KMnO_4$) in a beaker of still water. Do not move or stir the water during the experiment.

S 2. Measure the diameter of the initial circle of potassium permanganate on the bottom of the beaker. _____

T 3. Enter the time zero and the time required to diffuse across the bottom of the beaker.
S 4. Measure the distance moved (movement) (mm) and the final time, and calculate the speed of diffusion (mm/hr).
S 5. Enter all data in table 3.2 (fig. 3.9).

Figure 3.8 Screw cap test tube, dye, and gelatin. Dye placed at one end has diffused the distance indicated.

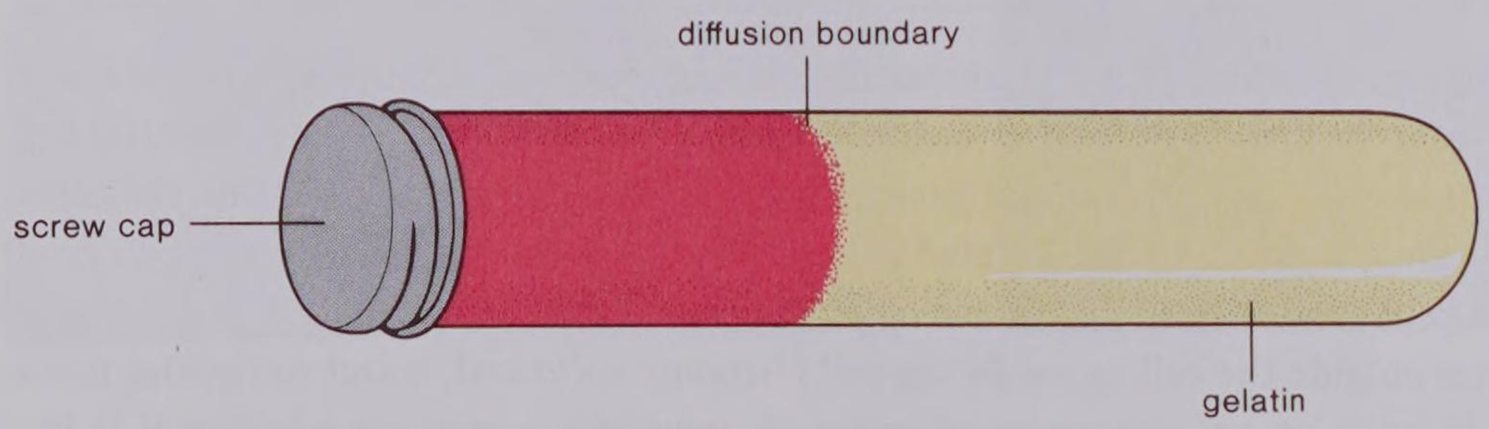
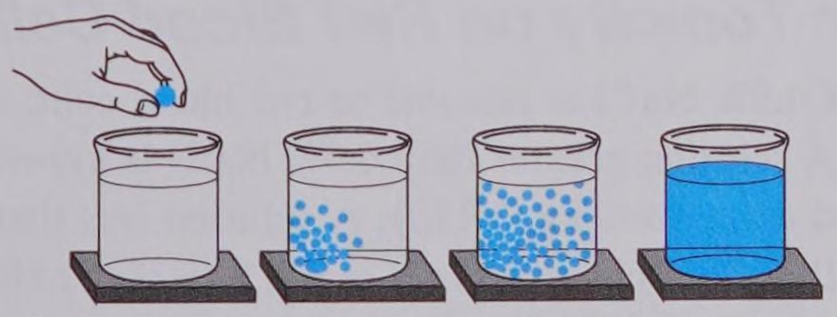


Figure 3.9 Diffusion is readily apparent when (***a***) perfume molecules fill a room or (***b***) water takes on the color of a dye.

a. **Diffusion in air**



b. **Diffusion in liquid**

Table 3.2 Speed of Diffusion

Exp. No.	Material	Time Zero	Final Time	Length of Time (hr)	Movement (mm)	Speed of Diffusion (mm/hr)
1	Dye in gelatin					
2	Spray in air					
3	Potassium permanganate in water					

In which experiment was diffusion the fastest? _____

In which was diffusion the slowest? _____

Movement of Molecules across Membranes

The plasma membrane, as well as artificial and animal membranes, is selectively permeable—it permits certain small molecules (water, ions, and small organic molecules) to diffuse across. These remaining exercises demonstrate a special kind of diffusion—*osmosis*—and the effect of solute (dissolved substance) concentration on the movement of water through a plasma membrane (a living selectively permeable membrane).

D *Osmosis* is the diffusion of water across a selectively permeable membrane. To demonstrate osmosis, a thistle tube was covered with a membrane at its lower opening and was partially filled with 50% corn syrup (Karo) or a similar substance. The whole apparatus was placed in a beaker containing distilled water (fig. 3.10). The concentration of water in the beaker is 100%.

1. Note the level of liquid in the osmometer tube and measure how far it travels in 10 minutes. _____ (mm)
2. Calculate the mm/hr that it would move _____. Water is a small molecule that readily passes through selectively permeable membranes from where it is highly concentrated to where it is less concentrated. The water concentration inside the tube is less because a given volume of corn syrup contains fewer water molecules than the same volume of distilled water. In which direction is there a net movement of water?

_____

3. Describe what you observed to support this statement. _____

_____

In this laboratory, we study the effects of diffusion of water across the plasma membrane (*osmosis*) in red blood cells and in *Elodea* cells. Water will diffuse from the area of its greater concentration to the area of its lesser concentration. If cells are placed in distilled water (*hypotonic solution*), the area of greater concentration of water is outside the cells, and water will diffuse *into* the cells. If cells are placed in a concentrated salt solution (*hypertonic solution*), the area of greater concentration of water is inside the cells, and water will diffuse *out* of the cells. If cells are placed in a solution that contains the same concentration of water outside the cell as inside the cell (*isotonic solution*), water molecules move back and forth across the membrane, but there is *no net* movement of water. A solution's *tonicity* is whether it is hypotonic, isotonic, or hypertonic.

Effect of Tonicity on Red Blood Cells (Animal Cells)

A solution of 0.9% NaCl is *isotonic* to red blood cells; in such a solution, the cells maintain their *normal* appearance (fig. 3.11*a*). A solution greater than 0.9% NaCl is *hypertonic* to red blood cells; in such a solution the cells shrivel up, a process called *crenation* (fig. 3.11*b*). A solution less than 0.9% NaCl is *hypotonic* to red blood cells; in such a solution the cells swell to bursting, a process called *hemolysis* (fig. 3.11*c*).

To observe the effect of solutions with various tonicities on red blood cells, prepare the tubes listed. State the tonicity of each tube in table 3.3 and fill in your results.

Figure 3.10 Osmosis demonstration. A thistle tube placed in distilled water contains sugar solution and is covered at one end by a membrane. As water enters the tube, the level of the solution rises.

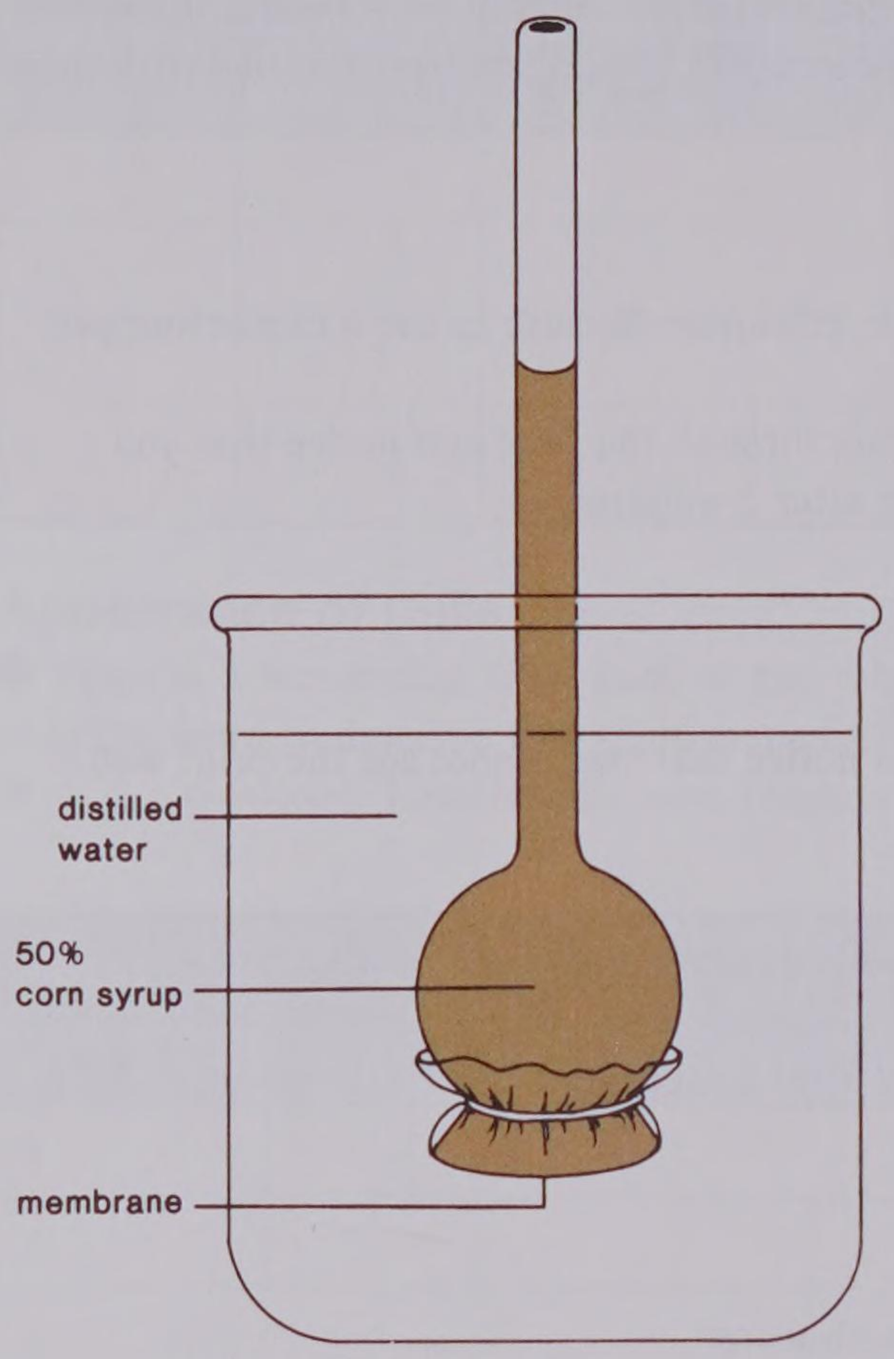
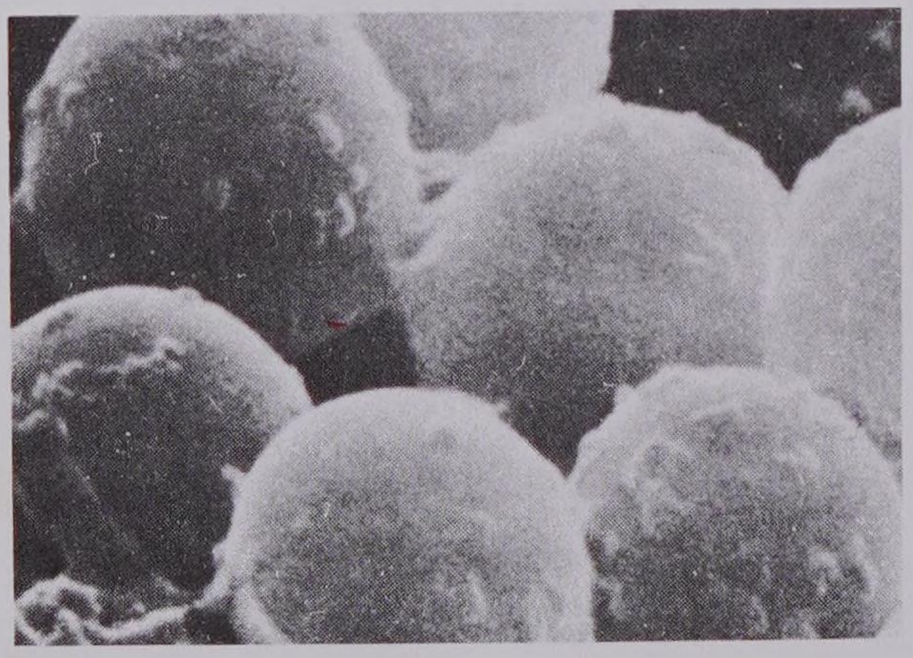


Figure 3.11 Red blood cells in (***a***) isotonic solution, (***b***) hypertonic solution, (***c***) hypotonic solution. (Photos courtesy of S. J. Singer.)

a.

b.



c.

Experimental Procedure

Sheep blood used in this experiment is obtained from a biological supply company that aseptically obtained, but did not sterilize, the blood from living sheep. Even though the health risk is negligible, one should always handle blood with *caution!! Do not touch the blood.* Be sure the test tubes have screw caps or stoppers. When you have finished with these test tubes, handle them as described by your instructor.

Tube 1

S 1. With a ruler, mark off the tube at 5 cm.
S 2. Fill to the mark with *0.9% NaCl* and add 3 drops of *blood*. Shake. (*Caution:* Be sure to use a cap or stopper. *Do not* cover the opening of the tube with your thumb.)
S 3. Place the tube in front of your manual as shown in figure 3.12. Look through the tube and notice that you cannot see the print well. (Note! Some tubes will never clear. Stop after 5 minutes.)

Tube 2

S 1. Mark off the tube at 5 cm.
S 2. Fill to the mark with *10% NaCl* and add 3 drops of *blood*. Shake.
S 3. Place the tube in front of your laboratory manual as before. Again notice that you cannot see the print well.

Tube 3

S 1. Mark off the tube at 2 cm and 5 cm.
S 2. Fill to the first mark with *0.9% NaCl* and to the second mark with *water.*
S 3. Add 3 drops of *blood* and shake.
T S 4. Place the tube in front of your manual and note the time for the print to appear.
S 5. Record your results.

Tube 4

S 1. Mark off the tube at ½ cm and 5 cm.
S 2. Fill to the first mark with *0.9% NaCl* and to the second mark with *water.*
S 3. Add 3 drops of *blood* and shake.
T S 4. Place the tube in front of your manual and note the time for the print to appear.
S 5. Record your results.

Figure 3.12 Proof of hemolysis.

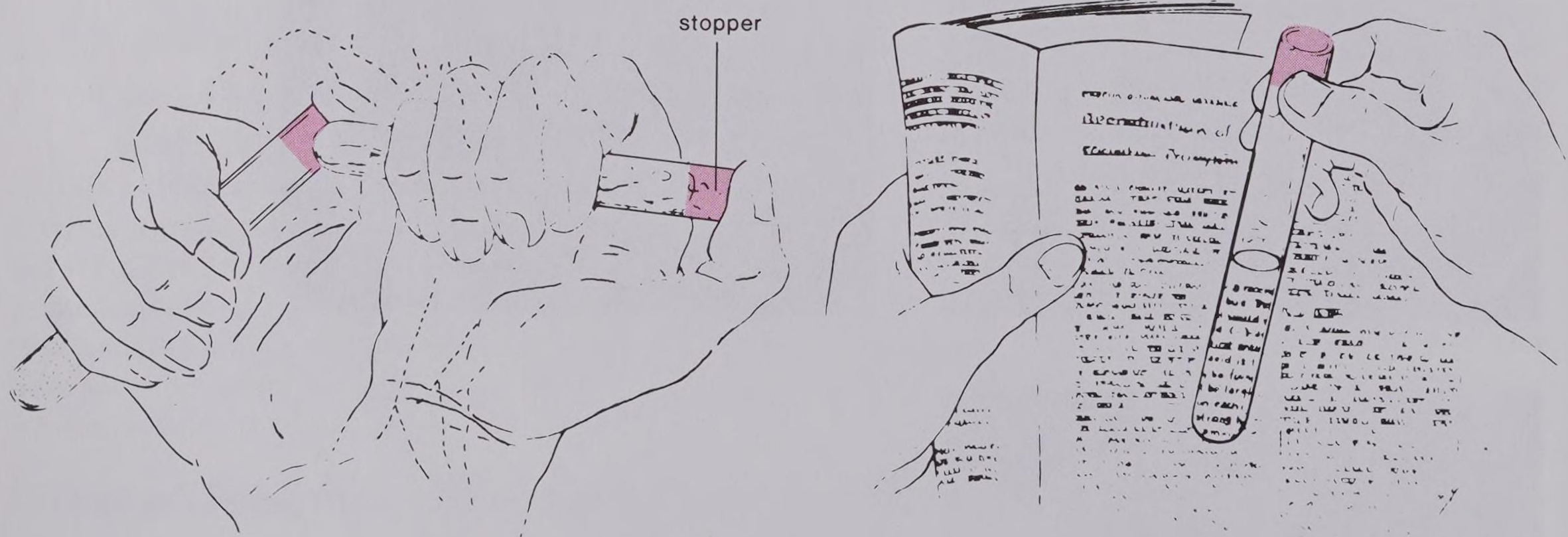


Table 3.3	Red Blood Cell Observation			
Tube No.	**Contents**	**Relative Tonicity (hypo-, hyper-, isotonic)**	**Time to See Print (sec)**	**Explanation**
1				
2				
3				
4				

Appearance of Cells

Prepare a wet mount from each of the tubes, check the appearance of the cells, and in table 3.4 give the correct scientific word to describe this appearance (refer to fig. 3.1).

Caution!! Handle with care. Dispose of slides as directed by your instructor.

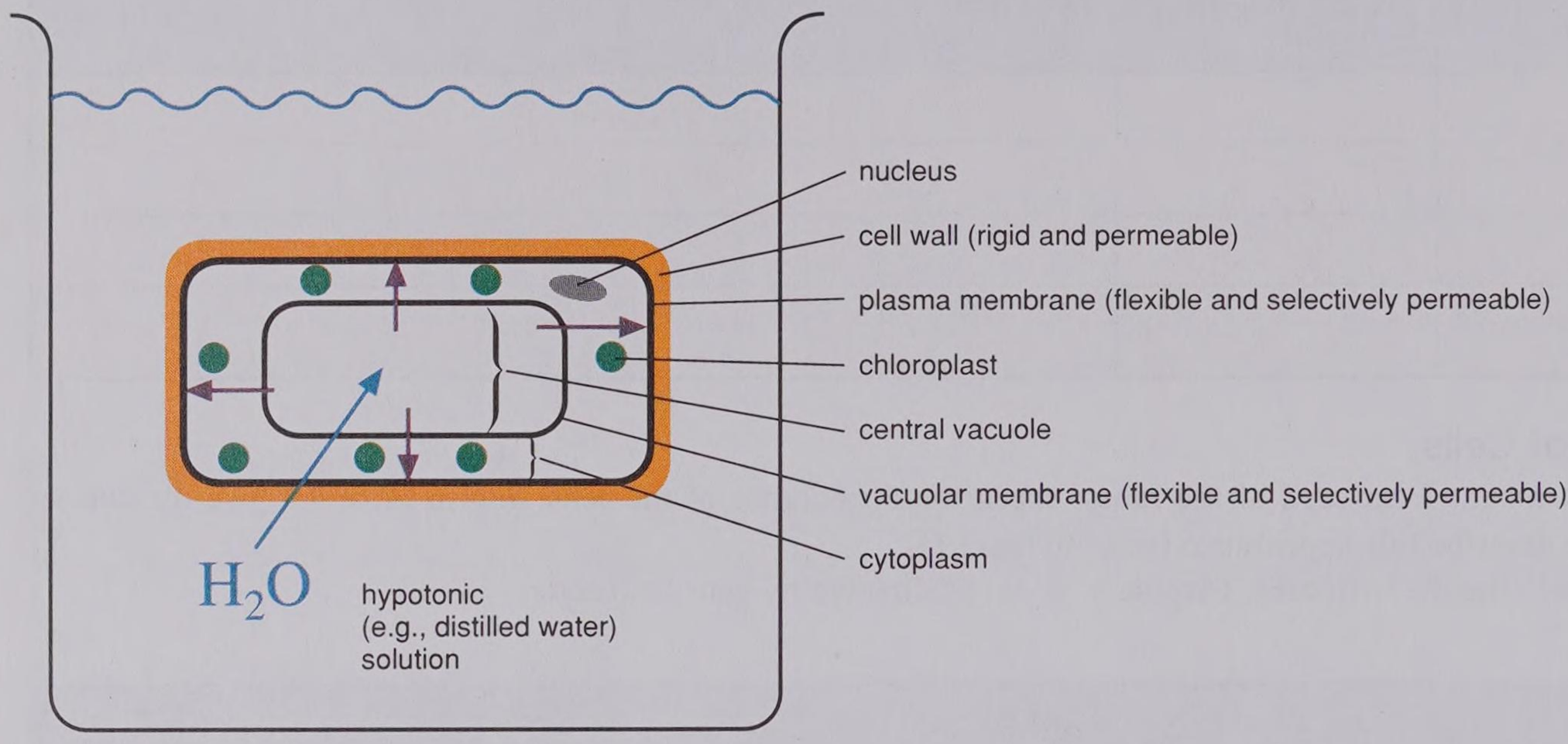
Table 3.4	Effects of Tonicity on Red Blood Cells		
Tube No.	**Tonicity**	**Appearance of Cells**	**Scientific Term**
1			
2			
3			
4			

Effect of Tonicity on Elodea *Cells (Plant Cells)*

When an *Elodea* plant is placed in distilled water, a hypotonic solution, the large, membrane-bounded central vacuole gains water and exerts pressure (*turgor pressure*) that compresses the cytoplasm (containing the chloroplast) and plasma membrane against the cell wall (fig. 3.13*a*). If the microscope's plane of focus is through the center of the cell, the chloroplasts are located around the perimeter, and the central vacuole appears transparent. When the top or bottom surface of the cell is in focus, the chloroplast appears uniformly distributed throughout the cell (fig. 3.13*b*).

1. Observe the *Elodea* slide you prepared near the beginning of this laboratory and note its appearance.
2. Be sure to use your fine focus first to view the top surface of the cell (fig. 3.13*b*), then focus downward to view the central portion of the cell.
3. Record its appearance in table 3.5.

Figure 3.13 *Elodea* cells placed in a hypotonic solution have a high internal pressure (turgor pressure) so that the plasma membrane and cellular contents push firmly against the cell wall. ***a.*** Diagrammatic representation of the net flow of water *into* the cell. ***b.*** Photograph of an *Elodea* cell with the upper surface in focus.



a.

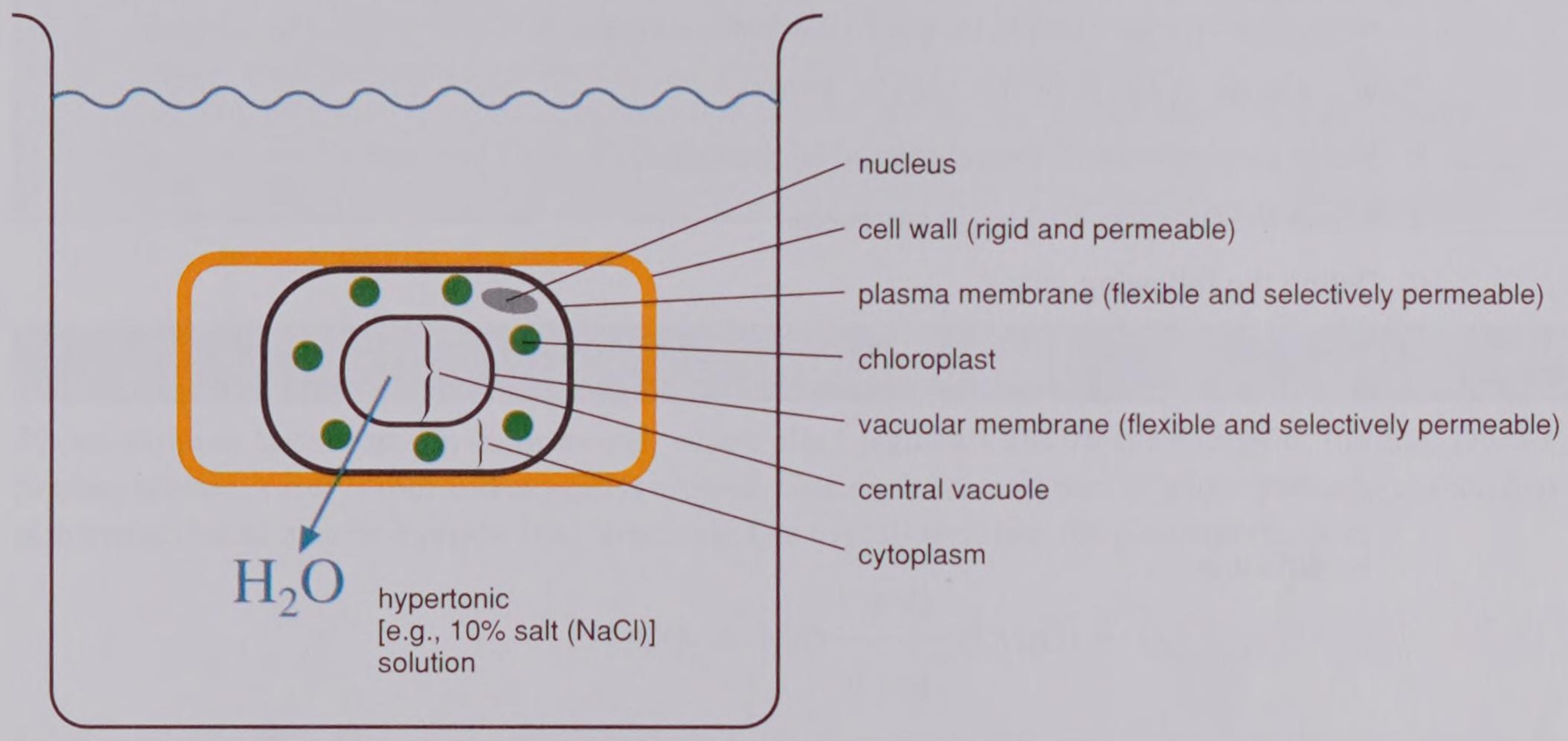
b.

Table 3.5	***Effects of Tonicity on Elodea Cells***	
Solution	**Appearance of Cell (sketch)**	**Scientific Term**
Hypotonic		Normal
Hypertonic		

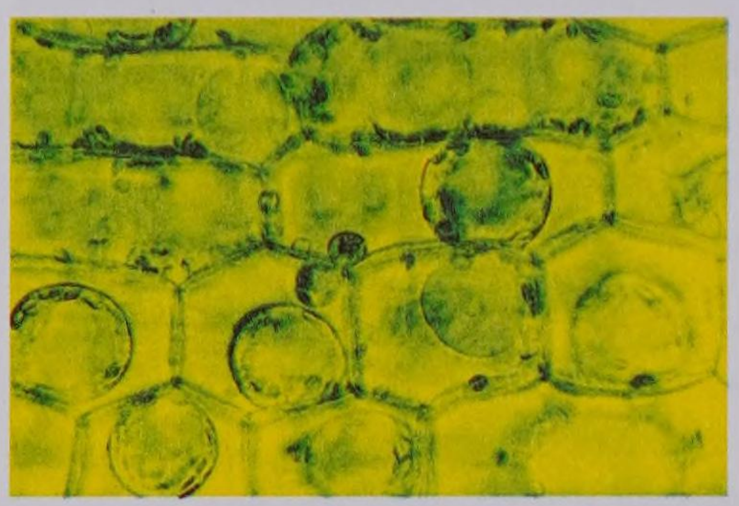
When an *Elodea* plant is placed in a hypertonic solution (10% salt [NaCl]), water moves out of the large, membrane-bounded central vacuole and cytoplasm into the solution. The volume of the cell is reduced and the plasma membrane is visibly pulled away from the cell wall (*plasmolysis*) (fig. 3.14). The water concentration inside the cell is higher than the water concentration outside of the cell. Molecules move from a region of their *high* concentration to a region of their *low* concentration. Therefore, the net flow of water is *out* of the cell.

S 1. Prepare a wet mount of a small *Elodea* leaf in a *10% salt* (NaCl) solution.
S 2. Set aside and observe after several minutes.
T S 3. Record its appearance in the second portion of table 3.5.

Figure 3.14 *Elodea* cells placed in a hypertonic solution have a net flow of water *outward* as shown by the reduction in the volume of the cellular contents and central vacuole. The plasma membrane is now visible surrounding the cellular contents in the center of the cell, a condition called plasmolysis. ***a.*** Diagrammatic representation of the net flow of water *out* of the cell. ***b.*** Photograph of an *Elodea* cell showing the withdrawn plasma membrane surrounding a smaller volume of cellular contents.



a.



b.

Laboratory Review 3

_____ 1. What is the function of the following organelles?

_____ a. mitochondria

_____ b. lysosomes

_____ c. smooth endoplasmic reticulum

_____ d. nucleoulus

_____ 2. Do plants and animals carry on cellular respiration?

_____ 3. Do both carry on photosynthesis?

_____ 4. What organelle is involved in respiration?

_____ 5. What organelle is involved in photosynthesis?

_____ 6. Name one type of vacuole that is found in the amoeba.

_____ 7. What is the function of this (#6) vacuole?

_____ 8. Name another type of vacuole found in the amoeba.

_____ 9. Name the function of this (#8) vacuole.

_____ 10. Define the following terms:

a. osmosis

b. diffusion

c. hemolysis

d. hypotonic medium

e. turgor pressure

Laboratory

4

Photosynthesis

Learning Objectives

Students should be able to

1. describe the separation of plant pigments by paper chromatography;
2. explain an experiment that indicates that white light promotes photosynthesis;
3. describe white light as composed of many colors of light;
4. explain the effectiveness of various colors of light for photosynthesis;
5. describe an experiment that indicates that carbon dioxide is utilized during photosynthesis;
6. describe the relationship between cellular respiration and photosynthesis.

Introduction

Photosynthesis takes place in chloroplasts, where plant pigments absorb the energy of the sun. During the first part of photosynthesis, water is split and oxygen is evolved. During the second part of photosynthesis, carbon dioxide is assembled into molecules of a carbohydrate (i.e., glucose). The overall equation for photosynthesis is:

$$CO_2 + H_2O \xrightarrow[\text{of sun}]{\text{energy}} (CH_2O) + O_2$$

In this equation (CH_2O) represents any carbohydrate. Sometimes this equation is multiplied by 6 so that glucose $C_6H_{12}O_6$ appears as an end product, as in the section titled "Carbon Dioxide Fixation" later in this laboratory.

Plant Pigments

The principal pigment in higher plants is chlorophyll *a*. Chlorophyll *b*, carotenes, and xanthophylls play a secondary role by transferring the energy they absorb to chlorophyll *a* for use in photosynthesis. In the following experiment, the pigments of a leaf are separated by means of paper chromatography.

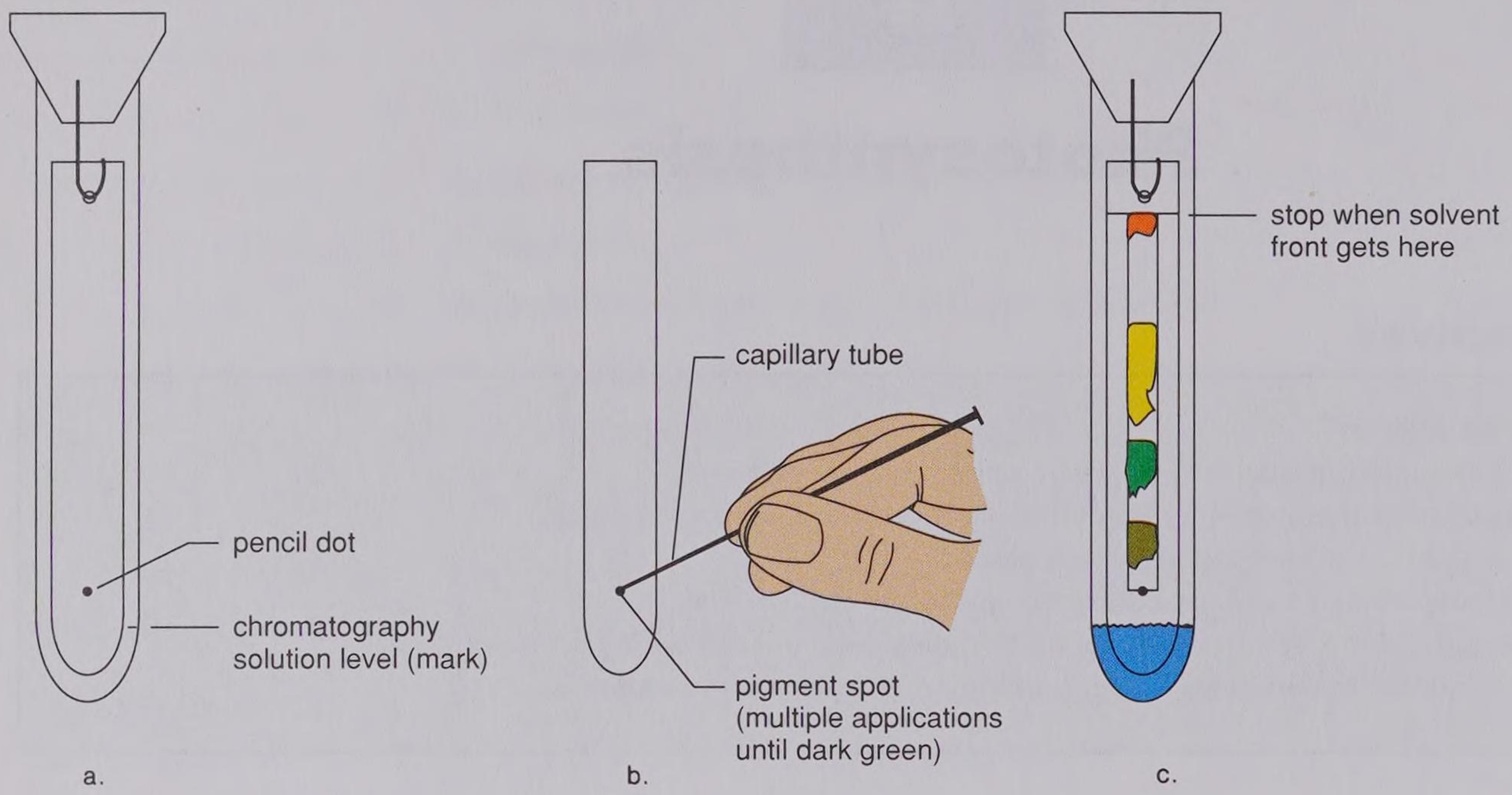
S 1. Obtain a chromatography apparatus (large dry test tube and cork with a hook) and a strip of precut chromatography paper (handle only by the top and edge).

S 2. Place the paper strip onto the hook and test for fit. The paper should hang straight and not rest on the bottom of the test tube; trim if necessary.

S 3. Measure 2 cm from the bottom of the paper and place a small dot with a pencil (not a pen).

S 4. Mark the test tube 1 cm below where the dot is with the stopper in place. Set the apparatus into a test tube rack while preparing the pigment extract (fig. 4.1).

Figure 4.1 Paper chromatography apparatus. ***a.*** Cut paper to size—must hang and not touch sides of a dry tube. ***b.*** Apply multiple applications (30–50) of chlorophyll solution. ***c.*** Place in test tube filled to mark with chromatography solution and allow to develop.



Experimental Procedure

S 1. Prepare (or obtain) a *pigment* extract as directed by your instructor.

S 2. Place the premarked chromatography paper strip onto a paper towel.

S 3. Fill a capillary tube by placing it into the extract. (It will fill by its own capillary action.)

S 4. Repeatedly apply the *pigment* extract to the chromatographic strip. Try to obtain a small, dark green spot.

S 5. In a fume hood, fill the test tube to the mark with *chromatography* solution and then carefully assemble your chromatography apparatus. Set into a test tube rack. *Caution:* Ether is toxic and extremely flammable. Do not breathe the fumes, and do not place the chromatography jar near any source of heat.

T 6. Allow 10–20 minutes for your chromatogram to develop. The pigments (chlorophylls *a* and *b,* xanthophyll, and carotene) will separate from each other based on their relative solubilities in the solvent. The chromatography solution contains 2 *nonpolar* solvents, petroleum ether and acetone. These solvents are bonded by covalent bonds that share their electrons fairly equally so that the molecule is not charged. (Water, a common solvent, shares electrons unequally such that the oxygen end is negative and the hydrogen end is positive. Such molecules are called *polar* molecules.) As the nonpolar solvent moves up the chromatography paper, the pigments move along with it. The more nonpolar the pigment, the more soluble it is in the nonpolar organic solvent, the faster it will move and the greater distance it will proceed up the paper.

Results

S 1. When the solvent front has moved within 1 cm of the upper edge of the paper, remove your chromatogram and mark the location of the solvent front lightly with a pencil and allow to dry in the hood.

S 2. Identify the pigment bands. The beta-carotenes will be the bright orange-yellow band at the top; xanthophylls are yellow in color and there may be multiple bands of xanthophylls. The uppermost, blue-green band is chlorophyll *a,* and the lowest, olive-green band is chlorophyll *b*. Which pigment was the most nonpolar (i.e., which had the greatest affinity for the nonpolar solvent)? _____

S 3. For the following calculations mark the center of the initial pigment spot; this will be the starting point for all the following measurements. Also mark the highest point of each pigment and the solvent front.

4. Place the measurements below and calculate the R_f (ratio-factor) values for each pigment by using the formula:

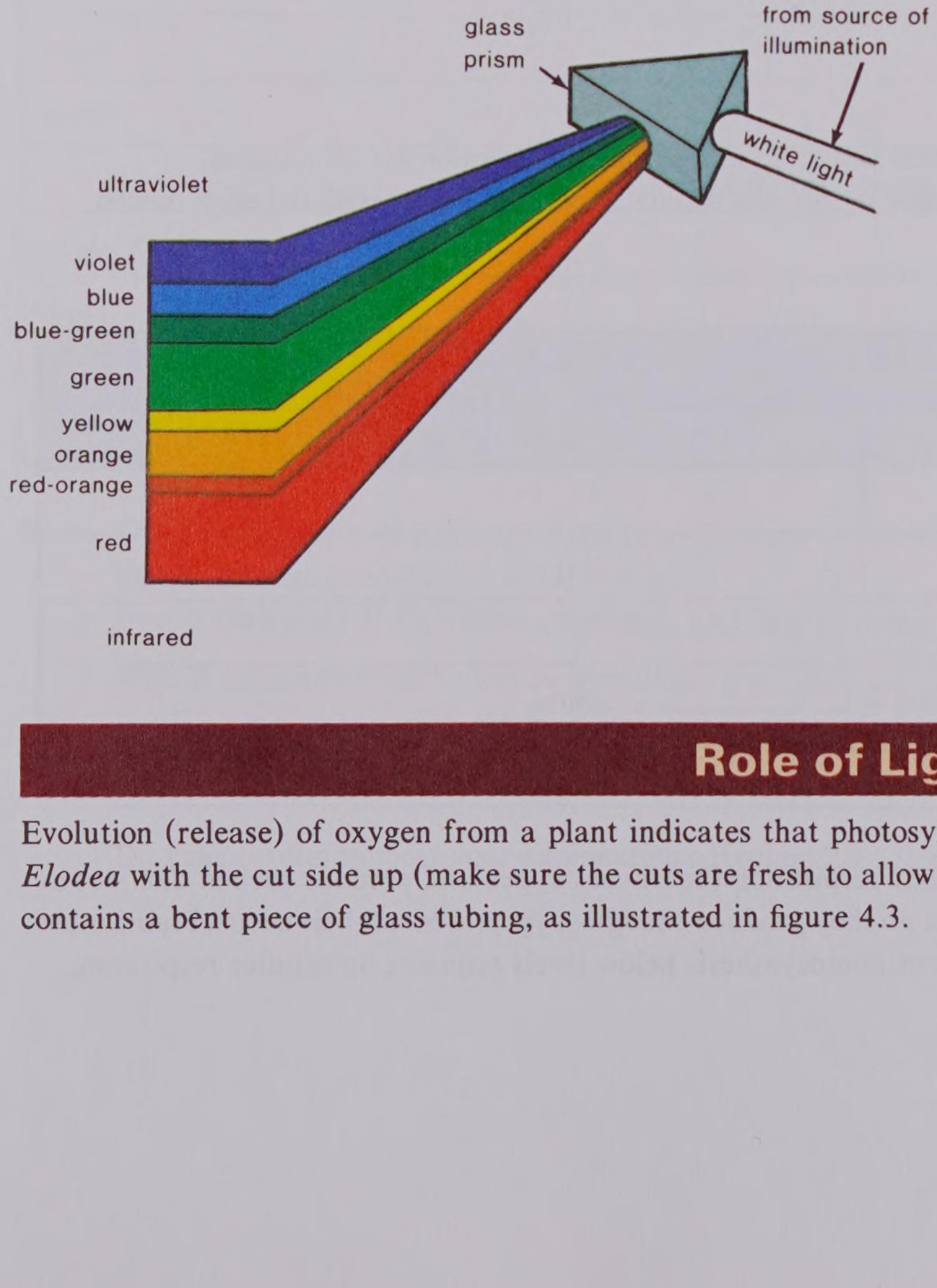
$$R_f = \frac{\text{distance moved by solute (pigment)}}{\text{distance moved by solvent}}$$

	Measurements (mm)	**R_f values**
Beta-carotenes	_____	_____
Xanthophylls	_____	_____
Chlorophyll *a*	_____	_____
Chlorophyll *b*	_____	_____

Do your results suggest that the chemical characteristics of these pigments might differ according to their color? _____ Materials differentially absorb (absent colors) and reflect (present colors) certain colors (wavelengths) of the visible spectrum to produce the colors we see.

5. Observe the colors of the full visible spectrum (demonstration) as produced by white light directed through a prism or diffraction grating. White (sun) light contains different colors of light, as can be seen when white light passes through a prism (fig. 4.2).
6. Use the information from the prism demonstration and figure 4.4 to answer the following questions. What color light do the chlorophylls absorb least? _____ How do you know? _____ What color light do the carotenes and xanthophylls absorb least? _____ Why is it beneficial to plant photosynthesis to have pigments of different colors? _____

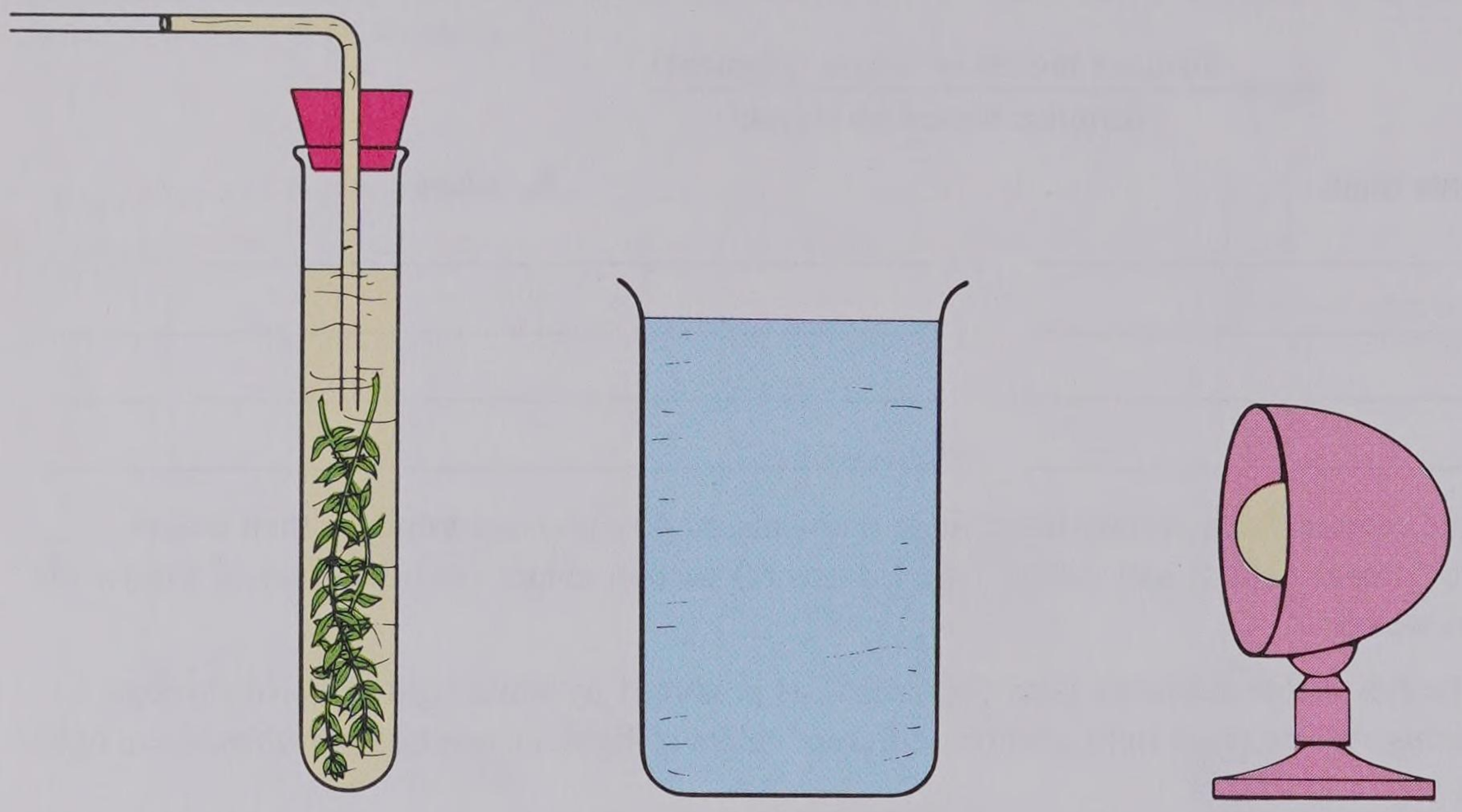
Figure 4.2 White light is made up of various colors, as can be shown when it is passed through a prism.



Role of Light

Evolution (release) of oxygen from a plant indicates that photosynthesis is occurring. Place a generous quantity of *Elodea* with the cut side up (make sure the cuts are fresh to allow escape of gasses) in a test tube whose rubber stopper contains a bent piece of glass tubing, as illustrated in figure 4.3.

Figure 4.3 Volumeter apparatus to study the need for light in photosynthesis.



Experimental Procedure

Ⓢ 1. Before stoppering, fill the tube with *3% aerated $NaHCO_3$ sodium bicarbonate* solution.
Ⓢ 2. Add sufficient solution so that when the rubber stopper is inserted into the tube, the solution comes to rest at about one-fourth the length of the bent glass tubing. Mark this location with a wax pencil.
Ⓢ 3. Place the tube in a test tube rack that will allow light to enter from the side.
Ⓢ 4. Place a beaker of plain *water* next to the *Elodea* tube. The water serves as a heat absorber. Next to the beaker place a lamp (150 watt). The tube, beaker, and lamp should be as close to one another as possible, plus the room light should be subdued.

White Light

Ⓣ 1. As soon as the edge of the solution in the side arm begins to move, time the reaction for 10 minutes.
Ⓢ 2. Again, mark the edge of the solution, and measure in mm the distance (net photosynthesis) the edge moved.
Ⓢ 3. Record your results in table 4.1.

Table 4.1 Rate of Photosynthesis

	Distance (mm) moved in 10 minutes (.167 hr)
White light (net photosynthesis)	+
No light (aerobic cellular respiration)	−
Total (gross photosynthesis)	
Rate of photosynthesis: gross photosynthesis ÷ time (.167 hr) = _____ mm/hr	

Respiration (Aerobic) in Plants

Plants contain mitochondria and undergo aerobic cellular respiration (consume oxygen and produce carbon dioxide) as long as they are alive. Only when there is sufficient light do they produce enough oxygen to meet their needs. Plants must consume outside oxygen when low light intensity reduces photosynthesis below levels required by cellular respiration.

No Light

T S 1. Wrap the tube containing *Elodea* in aluminum foil. Wait 10 minutes and measure the distance the edge has receded.

S 2. Record your results in table 4.1. Considering the fact that a plant is continually undergoing aerobic cellular respiration (in its mitochondria), why did the edge recede? _____ If the *Elodea* had not been respiring when the lamp was on, how far would the edge have moved in the preceding experiment? ____

S 3. The total amount of oxygen actually produced (gross photosynthesis) by the plant is the total of both the forward and reverse movement of the liquid. Place this total in table 4.1. Calculate the rate of photosynthesis by dividing the total (gross photosynthesis) by the time (.167 hr = 10 min). Place the results in table 4.1.

Green Light (Optional)

S 1. Add *green food coloring* to the water in your beaker until there is a distinctive color.

S 2. Remove all previous wax pencil marks on the side arm and mark the present edge of the solution.

S 3. Turn on the lamp.

T S 4. Wait 10 minutes and measure the distance moved in mm.

S 5. Record your results in table 4.2.

Comparison

S 1. Record in table 4.2 the results obtained by other groups in the class.

Table 4.2 Green Light Experiment

Color	Distance Moved	
	Your Data	Other Data
White		
Green		
Rate of photosynthesis (mm/hr)		
White		
Green		
Percentage (your data) = $\frac{\text{Rate of photosynthesis (green light)}}{\text{Rate of photosynthesis (white light)}} \times 100 =$		

S 2. Calculate the rate of photosynthesis in green light as previously described for white light, and then calculate the percentage as indicated in table 4.2.

3. Recall the colors of the visible spectrum, and look at figure 4.4 to answer the following questions.

Should photosynthesis be less in green light? _____

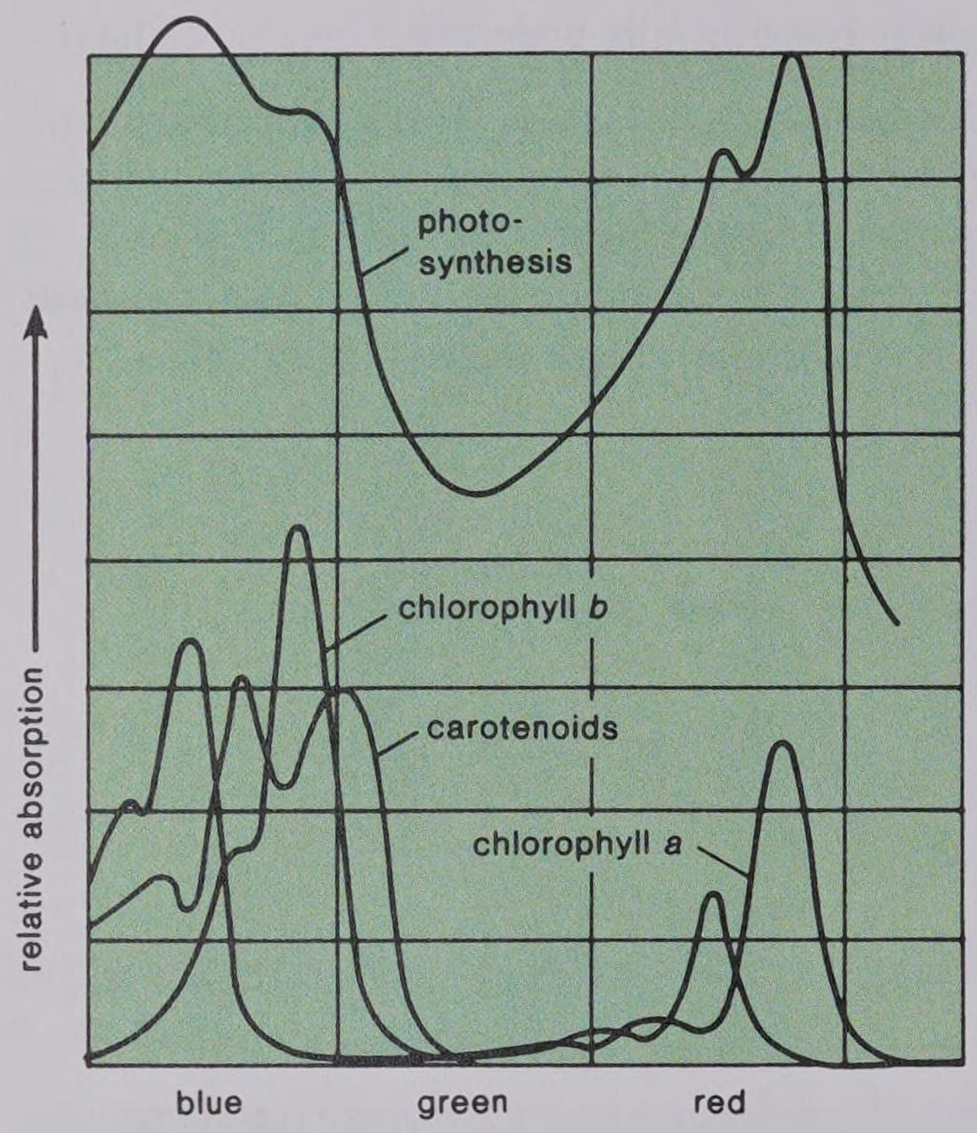
Explain why or why not? _____

_____

Do your results support your explanation? Explain _____

_____

Figure 4.4 Absorption spectrums for individual pigments (*bottom*) and photosynthesis (*top*). Each pigment absorbs light rays as indicated to produce the absorption spectrum for photosynthesis. Carotenes and xanthophylls belong to a class of pigments called carotenoids.



Carbon Dioxide Fixation

During the second stage of photosynthesis, carbon dioxide is taken up by the plant, and along with available hydrogen (from water), it is assembled into a carbohydrate (CH_2O) such as glucose $C_6H_{12}O_6$.

Experimental Procedure

S 1. Temporarily remove the *Elodea* and rinse with *water*. Discard the contents of the tube, *sodium bicarbonate,* and rinse the tube with *water*.

S 2. Fill the test tube with a dilute solution of *phenol red* adjusted to a delicate pink. Phenol red is a pH indicator that turns yellow in acid and red in base.

S 3. Using an individually wrapped straw, bubble breath into the solution until the color changes to yellow.

Breathing into the solution by means of the straw adds what gas to the test tube? _____

When carbon dioxide combines with water, it forms carbonic acid. What causes the color change? _____

_____

S 4. Return the *Elodea* to this test tube and assemble your equipment as before.

T S 5. Turn on the light and wait until the edge of the solution just begins to move. Note the time and turn off the light. Do this repeatedly until you note a change in color.

S 6. Record your results in table 4.3. Explain why the solution in the test tube eventually turns red. _____

_____

Table 4.3 Carbon Dioxide Uptake

Test Tube	Time for Color Change
Experimental: *Elodea* + CO_2 + phenol red	
Control: _____ + _____	

Scientific Method (Optional)

Scientists perform experiments to support or deny a hypothesis, which is a statement or belief about the phenomenon being investigated. State a hypothesis to explain why the solution in the test tube turned red.

Hypothesis: _____

_____

How did your results support the hypothesis? _____

_____

_____

_____

Scientists are more confident of their results when the experimental procedure has included a control. Controls often include all of the experimental conditions and remove only the experimental variable. Considering your hypothesis, what ingredients would be appropriate for a control?

Control: _____

_____

Ask your instructor if you should actually perform this procedure; if so record your results in Table 4.3.

Why should all experiments have a control? _____

_____

Carbon Cycle

In this experiment, you have demonstrated a relationship between cellular respiration and photosynthesis. You produced the carbon dioxide that was used by the plant to carry out photosynthesis. In return, the plant will produce the food and oxygen you require to carry out cellular respiration. This relationship is illustrated in figure 4.5 and can be represented by this equation:

$$C_6H_{12}O_6 + 6O_2 \underset{\text{photosynthesis}}{\overset{\text{cellular respiration}}{\rightleftharpoons}} 6CO_2 + 6H_2O + \text{energy}$$

Which organelle in plants carries out this reaction in the reverse (right to left) direction? _____

During photosynthesis, the energy in this equation is _____.

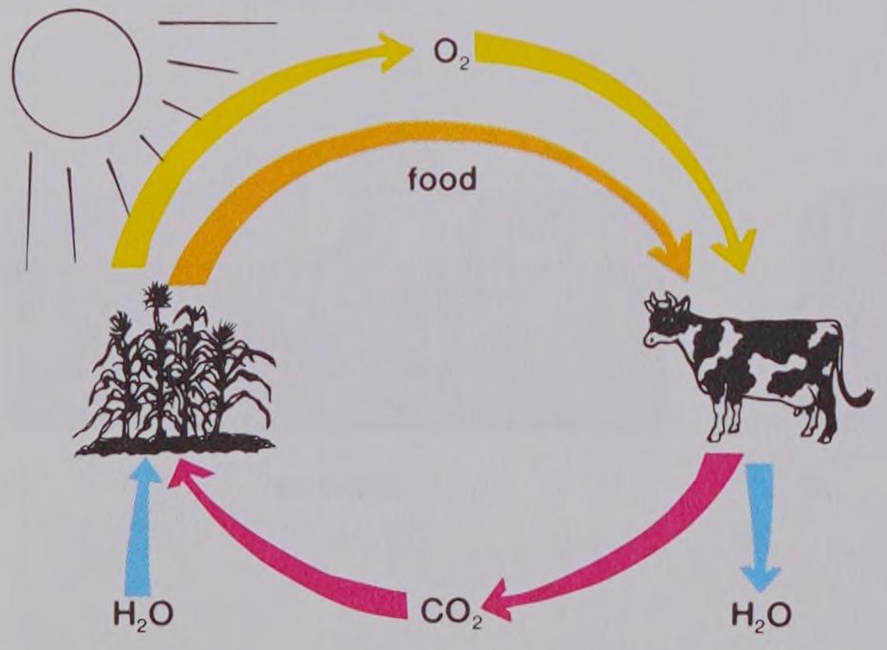
Which organelle in plants and animals is involved in carrying out this reaction in the forward (left to right) direction?

_____

During cellular respiration, the energy in this equation is _____.

Would it be correct to say that the energy of sunlight eventually becomes ATP energy? _____

Figure 4.5 Relationship between photosynthesis and respiration. Animals are dependent on plants for a supply of oxygen, and plants are dependent on animals for a supply of carbon dioxide.



Laboratory Review 4

_____ 1. What color is the phenol red solution when carbon dioxide is added?

_____ 2. Is the pH lowered or raised when carbon dioxide is added?

_____ 3. If you covered 3 leaves on a plant with aluminum foil and exposed the rest to light, would the exposed or covered leaves test positive for starch? Explain why.

_____ 4. Which pigment is the most nonpolar on your chromatogram?

_____ 5. What is the relationship between photosynthesis and cellular respiration?

_____ 6. Describe the process used to separate the different types of pigments found in plant tissues.

Laboratory

5

Enzymes

Learning Objectives

Students should be able to

1. discuss the function of an enzyme, and explain the role of the active site in enzyme specificity;
2. predict the effects of temperature, enzyme concentration, substrate concentration, pH, and inhibitors on enzyme reactions.

Introduction

Enzymes are proteins that speed up chemical reactions because they have a 3-dimensional shape that accommodates their *substrate(s),* the reactant(s) in the enzymatic reaction (fig. 5.1). This shape, therefore, determines the specificity of the enzyme and is important to the action of the enzyme because the reaction occurs at the region of contact between the substrate(s) and enzyme. This region is called the *active site.*

The specificity of an enzyme can be discussed in reference to the following equation:

enzyme + substrate—enzyme-substrate complex—enzyme + product 1 + product 2

or

$$E + S \rightarrow ES \rightarrow E + P_1 + P_2$$

Figure 5.1 An enzyme has a particular shape and a particular region, called the active site, where the reaction takes place. After the reaction, the enzyme and products separate.

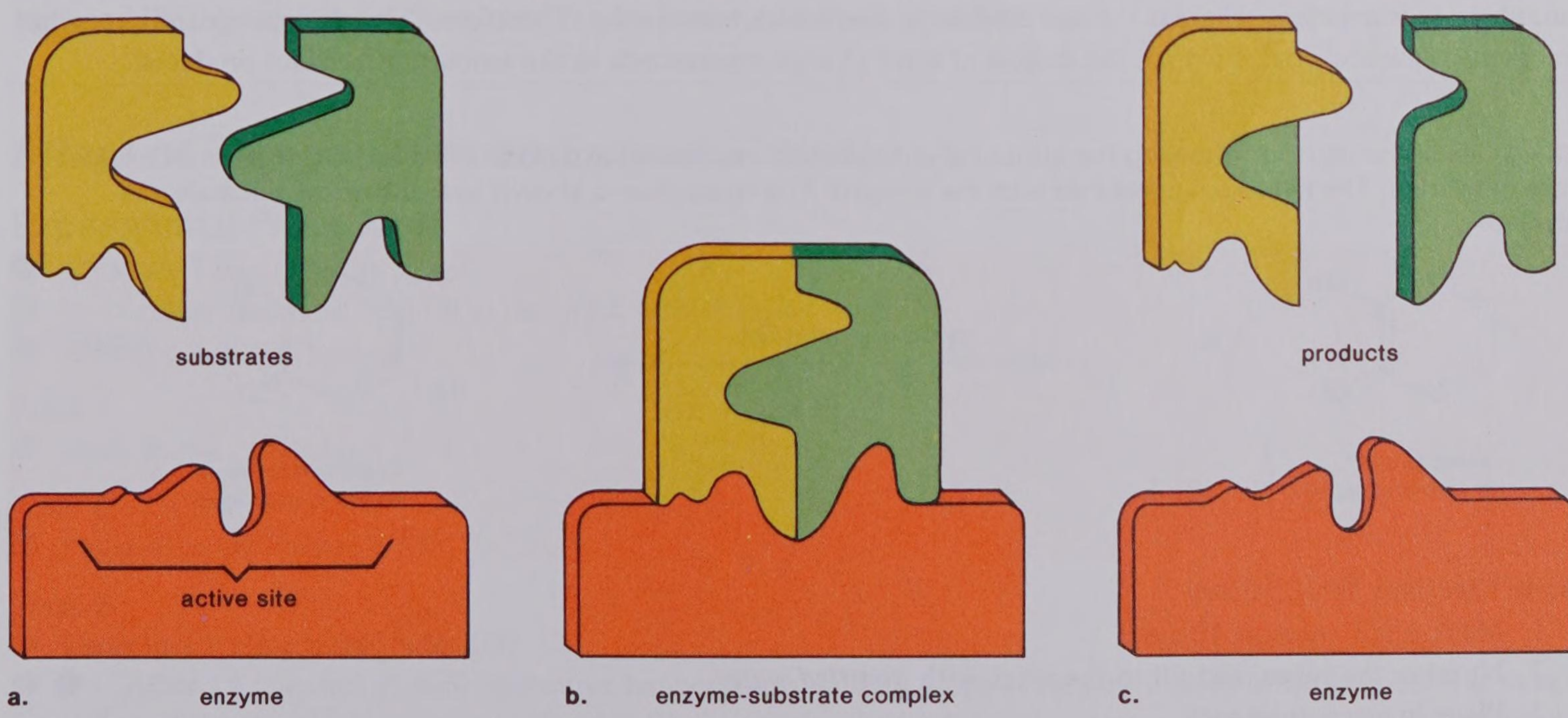
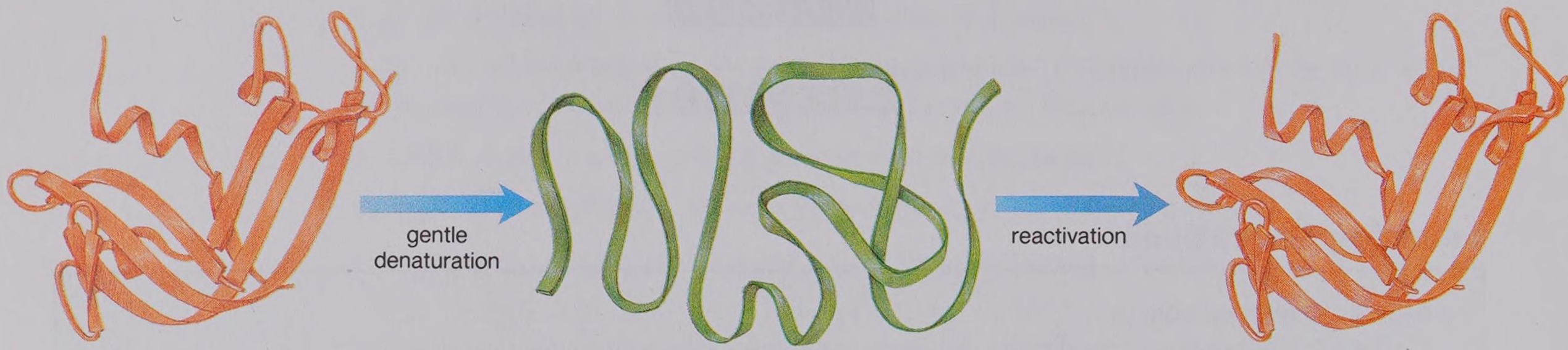


Figure 5.2 Denaturation and reactivation of the enzyme ribonuclease. When a protein is denatured, it loses its normal shape and activity. If denaturation was gentle and the conditions are removed, some proteins will regain their normal shape. This shows that the normal conformation of the molecule is due to the various interactions between a set sequence of amino acids. Each type of protein has a particular sequence of amino acids.



Notice that in this equation the substrate (S) becomes the products (P_1 and P_2), but enzyme is unchanged. This means that only a small amount of enzyme is actually needed in a cell because enzymes are used over and over again. Some enzymes have turnover rates well in excess of 1,000,000 substrate molecules per minute.

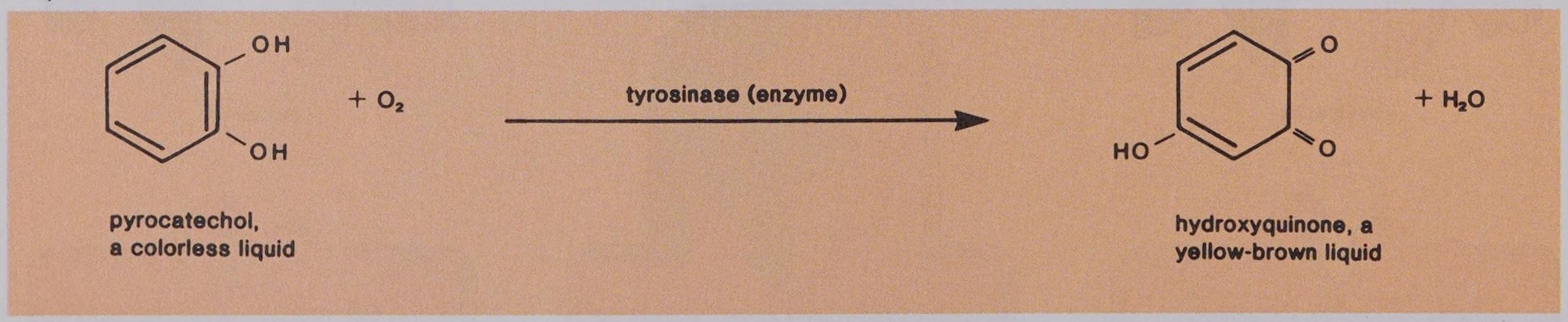
All enzymes are complex proteins that generally act in the closely controlled internal environment of an organism, where the temperature and pH remain within a rather narrow range. Extremes in pH or temperature may either temporarily inhibit the reaction or they may denature the enzyme by permanently altering its chemical structure. Even a small change in the tertiary or quaternary structure (fig. 5.2) will change the shape of the enzyme enough to prevent formation of the enzyme-substrate complex and thus prevent the reaction. (See your text and Laboratory 2 for more information on the structure of proteins.)

Sometimes another molecule is so close in shape to the enzyme's substrate that this molecule can compete with the true substrate for the active site of the enzyme. Every time this molecule combines with the enzyme, no product occurs; therefore, there is less product per unit time. Such a molecule is called an *inhibitor*.

Tyrosinase

Tyrosinase is an enzyme (found in many cells, including potato cells) that speeds up the reaction shown in figure 5.3. The substrate is colorless, whereas the end product is a yellowish brown color. Therefore, this color change indicates that the reaction has occurred. Further, the degree of color change corresponds to the amount of product produced.

Figure 5.3 The equation showing the structural change from the substrate pyrocatechol in the presence of oxygen hydroxyquinone. The hydrogen combines with the oxygen. The tyrosinase is shown over the arrow because it is recycled.



Experimental Procedure

- (S) 1. Mark 3 test tubes at 10 cm.
- (S) 2. Number the tubes, and fill to the mark with *distilled water.*
- (S) 3. Place in a test tube rack.

Tube 1

(S) 1. Add 10 drops of *enzyme* (tyrosinase) and 10 drops of *substrate* (pyrocatechol).
(S) 2. Shake the tube sideways several times and observe for 5 minutes.
(S) 3. Record the beginning color in table 5.1.
(T) (S) 4. Observe the tube for 5 minutes.
(S) 5. Record the color change. This is the standard end color to be expected for this reaction.

Tube 2

(S) 1. Add 10 drops of *substrate* (pyrocatechol) only.
(S) 2. Shake the tube, and then record the beginning color in table 5.1.
(T) (S) 3. Observe for 5 minutes and record the end color.

Tube 3

(S) 1. Add 10 drops of *enzyme* (tyrosinase) and 10 drops of an alternate substrate (sucrose).
(S) 2. Shake the tube, and then record the beginning color in table 5.1.
(T) (S) 3. Observe for 5 minutes and record the end color.

For all experiments in this laboratory, compare your results with those around you. If others' data differs, enter it in parentheses. *Do not* change your data. Make your explanations based upon the introductory information in this laboratory, discussion with the instructor, the reactions of the majority of the class, or what theoretically should have happened, since sometimes chemicals deteriorate or mistakes are made in mixing.

Table 5.1 Observations

Tube	Contents	Beginning Color	End Color	Explanation
1				
2				
3				

Which of these tubes could be called a control? _____

Which demonstrates enzyme specificity? _____

Explain how this experiment shows that enzymes are specific to a substrate? _____

_____

Effect of Temperature on Enzyme Activity

Experimental Procedure

(S) 1. Mark 3 test tubes at 10 cm.
(S) 2. Number the tubes, and fill to the mark with *distilled water.*
(S) 3. Place in a test tube rack.

Tube 1

(S) Place in the refrigerator, 5° C.

Tube 2

(S) Place in an incubator, 37° C.

Tube 3

(S) Place in a boiling *water bath,* 100° C.
(T) (S) After 15 minutes, at their respective temperatures, add 10 drops of *enzyme* and 10 drops of *substrate* to each tube. Record the beginning color for each tube. Observe each tube for 5 minutes. Note the end colors and record in table 5.2.

Table 5.2 Effect of Temperature				
Tube	**Temperature**	**Beginning Color**	**End Color**	**Explanation**
1	Refrigerator			
2	Incubator			
3	Boiling water			

Which tube shows the fastest reaction rate? Why? _____

_____

Effect of Enzyme Concentration on Enzyme Activity

Experimental Procedure

Ⓢ 1. Mark 3 test tubes at 10 cm.
Ⓢ 2. Number the tubes, and fill to the mark with *distilled water.*
Ⓢ 3. Place in a test tube rack.

Tube 1

Ⓢ 1. Add 3 drops of *enzyme* and 10 drops of *substrate.*
Ⓢ 2. Shake and record the beginning color in table 5.3.
Ⓣ Ⓢ 3. Wait 5 minutes and record the end color.

Tube 2

Ⓢ 1. Add 9 drops of *enzyme* and 10 drops of *substrate.*
Ⓢ 2. Shake and record the beginning color.
Ⓣ Ⓢ 3. Wait 5 minutes and record the end color.

Tube 3

Ⓢ 1. Add 27 drops of *enzyme* and 10 drops of *substrate.*
Ⓢ 2. Shake and record the beginning color.
Ⓣ Ⓢ 3. Wait 5 minutes and record the end color.

Table 5.3 Concentration of Enzyme				
Tube	**Amt. of Enzyme**	**Beginning Color**	**End Color**	**Explanation**
1				
2				
3				

Which tube exhibited the fastest reaction rate? _____

Explain. _____

_____

Effect of Substrate Concentration on Enzyme Activity

Experimental Procedure

S 1. Mark 3 test tubes at 10 cm.
S 2. Number the tubes, and fill to the mark with *distilled water.*
S 3. Place in a test tube rack.

Tube 1

S 1. Add 3 drops of *substrate* and 10 drops of *enzyme.*
S 2. Shake and record the beginning color in table 5.4.
T S 3. Wait 5 minutes and record the end color.

Tube 2

S 1. Add 9 drops of *substrate* and 10 drops of *enzyme.*
S 2. Shake and record the beginning color.
T S 3. Wait 5 minutes and record the end color.

Tube 3

S 1. Add 27 drops of *substrate* and 10 drops of *enzyme.*
S 2. Shake and record the beginning color.
T S 3. Wait 5 minutes and record the end color.

Table 5.4 Concentration of Substrate

Tube	Amt. of Substrate	Beginning Color	End Color	Explanation
1				
2				
3				

Which tube exhibited the fastest reaction rate? _____

Explain._____

_____

Effect of pH on Enzyme Activity

Experimental Procedure

S 1. Mark 3 test tubes at 10 cm.
S 2. Number the tubes, and place in a test tube rack.

Tube 1

S 1. Fill to the mark with *water* adjusted to pH 3.
S 2. Add 10 drops of *enzyme* and 10 drops of *substrate.*
S 3. Shake and record the beginning color in table 5.5.
T S 4. Wait 5 minutes and record the end color.

Tube 2

S 1. Fill to the mark with water adjusted to pH 7.
S 2. Add 10 drops of *enzyme* and 10 drops of *substrate.*
S 3. Shake and record the beginning color.
T S 4. Wait 5 minutes and record the end color.

Tube 3

S 1. Fill to the mark with water adjusted to pH 11.
S 2. Add 10 drops of *enzyme* and 10 drops of *substrate.*
S 3. Shake and record the beginning color.
T S 4. Wait 5 minutes and record the end color.

Table 5.5 Effect of pH

Tube	pH of Tube	Beginning Color	End Color	Explanation
1	3			
2	7			
3	11			

What is the optimum pH for this enzyme? _____

Is there a pH at which this enzyme does not function at all? _____

or minimally functions? _____

Effect of Inhibitors on Enzyme Activity

Experimental Procedure

S 1. Mark 3 test tubes at 10 cm.
S 2. Number the tubes and fill to the mark with distilled water.
S 3. Place in a test tube rack.

Tube 1

S 1. Add 10 drops of *enzyme* and 10 drops of *substrate.*
S 2. Shake and record the beginning color in table 5.6.
T S 3. Wait 5 minutes and record the end color.

Tube 2

S 1. Add 10 drops of *enzyme* and 10 drops of *substrate.*
S 2. Add one drop of the inhibitor *phenylthiourea.*
S 3. Shake and record the beginning color.
T S 4. Wait 5 minutes and record the end color.

Tube 3

S 1. Add 10 drops of *enzyme* and 10 drops of *substrate.*
S 2. Add 20 drops of the inhibitor *phenylthiourea.*
S 3. Shake and record the beginning color.
T S 4. Wait 5 minutes and record the end color.

Table 5.6 Effect of Inhibitors

Tube	Amt. of Inhibitor	Beginning Color	End Color	Explanation
1				
2				
3				

What effect does the increase of the inhibitor have on enzyme action? _____

Inhibition of Cellular Respiration (Demonstration)[2]

Enzyme inhibitors act in a variety of ways. They bind to the enzyme so strongly that it is not available to act on the correct substrate (competitive inhibition), tie up an ingredient essential for the enzymes function (i.e., Mg ions) (non-competitive inhibition), or alter the specific structural nature of the protein denaturation.

In this experiment, yeast cells are placed in respirometer tubes that retain the carbon dioxide given off. Does this indicate that yeast cells are living and capable of carrying on cellular respiration? _____

Sodium fluoride (a poisonous substance that will be handled only by your instructor) inhibits (noncompetitively) enolase, an enzyme necessary to cellular respiration. Sodium fluroide (NaF) has been added to one respirometer. Do you expect this tube to show cellular respiration? _____ Why not? _____

_____

Experimental Procedure

Ⓓ Three respirometers have been assembled and filled with these solutions:

Tube 1
Water + yeast

Tube 2
Water + glucose + yeast

Tube 3
NaF + glucose + yeast

Ⓢ Ⓣ The degree of respiration is determined by the total amount of gas that has collected within 1 hour. At the end of this time, complete table 5.7.

Table 5.7 Inhibition of Cellular Respiration

Tube No.	Contents	Degree of Cellular Respiration	Explanation
1			
2			
3			

Do these results support the expectation that you stated previously? Explain. _____

Summary of Cellular Respiration and Its Products

The overall equation for cellular respiration is:

$$6\ O_2 + C_6H_{12}O_6 \longrightarrow 6\ CO_2 + 6\ H_2O$$

(oxygen) (glucose) (carbon dioxide) (water)

Answer the following questions, which are based on this equation. What is the beginning substrate for cellular respiration?

_____

What are the end products? _____

Considering your answers, what gas is given off while cellular respiration takes place? _____

In what organelle does cellular respiration occur? _____

[2]See the *Laboratory Resource Guide.*

Laboratory Review 5

_____ 1. What happens to the rate of enzyme reaction when you increase the concentration of an enzyme?

_____ 2. What is the effect of a change in pH (away from the preferred pH) on the rate of enzymatically catalyzed reactions?

_____ 3. How does boiling affect the rate of a reaction that is catalyzed by an enzyme?

_____ 4. What is denaturization?

pyrocatechol (substrate) + tyrosinase (enzyme) + O_2 $\longrightarrow$ pyrocatechol-tyrosinase (enzyme-substrate complex) $\longrightarrow$ hydroxyquinone (product) + H_2O (product) + tyrosinase (enzyme)

_____ 5. In the preceding reaction, identify the following:

_____ a. Substrates

_____ b. Enzyme-substrate complex

_____ c. Products

_____ 6. What do enzyme inhibitors do to reduce or stop the enzymatic reaction?

_____ 7. Why do organisms have narrow ranges of tolerance for changes in temperature and pH?

_____ 8. What gas is *produced* during the process of cellular respiration?

_____ 9. What gas is *consumed* during the process of cellular respiration?

References

Enger, Eldon, et al. 1985. *Concepts in Biology Laboratory Manual.* Dubuque, IA: Wm. C. Brown Publishers.

Laboratory

6

Mitosis and Meiosis

Learning Objectives

Students should be able to

1. name and identify the stages, the associated structures, and the events of mitosis using slides and models;
2. name and identify the stages, the associated structures, and the events of meiosis using slides and models;
3. compare mitosis to meiosis by listing 4 similarities and/or differences.

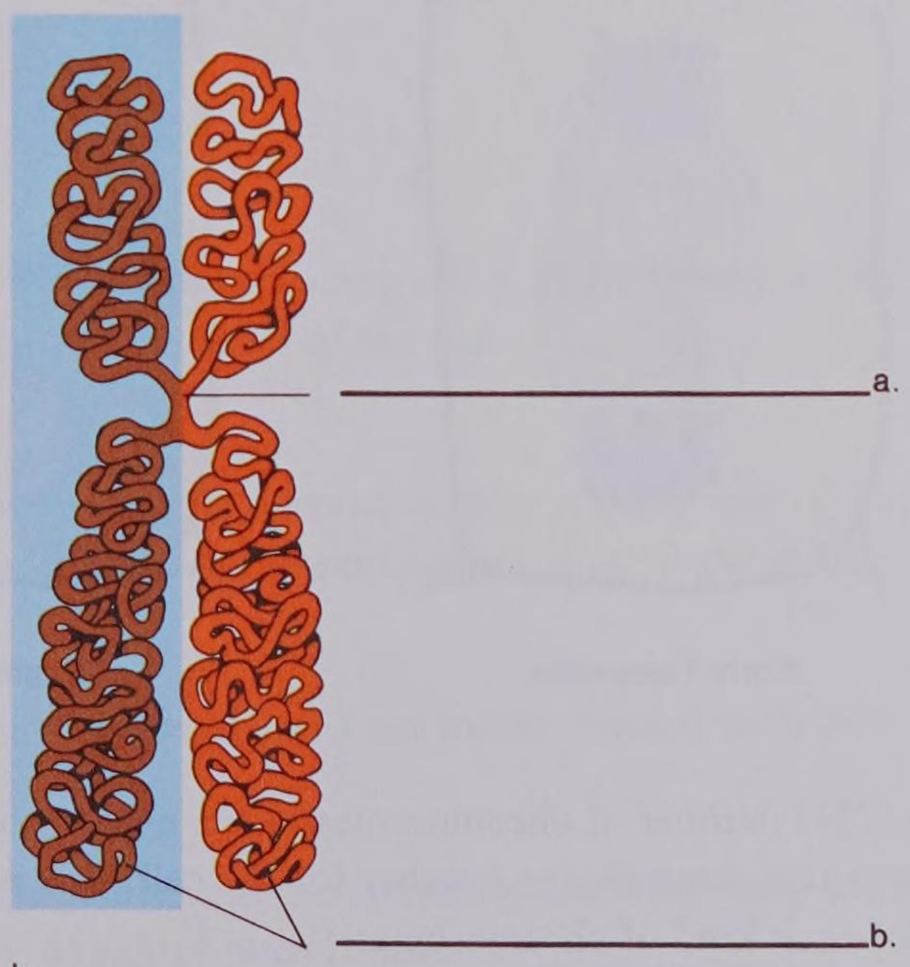
Mitotic Cell Division

Mitotic cell division is necessary to the growth and repair of multicellular organisms. It is also a form of asexual reproduction for eukaryotic single-celled organisms. Before dividing, a cell doubles all its components, including the organelles such as mitochondria, ribosomes, and centrioles if present. Because of DNA replication, each chromosome in the nucleus is made up of a pair of *sister chromatids* held together at the *centromere.*

Label one chromatid, sister chromatids, and the centromere in the drawing of a chromosome given in figure 6.1. This illustration represents a chromosome as it would appear just before nuclear division. *Mitosis* is nuclear division that results in 2 daughter nuclei, each with the same number and kinds of chromosomes as the nucleus of the mother cell. (The mother cell is the cell that divides; the daughter cells are the resulting cells.)

Figure 6.1 Duplicated chromosomes. DNA replication has occurred, and each chromosome is held together at a region called the centromere. ***a.*** Photomicrograph of highly coiled and compacted chromosome, typical of a nucleus about to divide. Magnification, ×8,400. ***b.*** Diagram of a chromosome that is not as condensed. One chromatid is indicated by a box. A duplicated chromosome contains sister chromatids, each containing copies of the same genes.

a.



b.

Mitosis Stages

Ⓢ With the aid of your text and available models, *label* the structures and stages described in figure 6.2.

Structures Associated with Plant Mitosis

The *nuclear envelope* is the double membrane that separates the nucleus from the cytoplasm. Visible within the nucleus during interphase are one or 2 oval *nucleoli,* which are concentrated regions of RNA, and *chromatin,* the grainy looking but actually threadlike strands of DNA, all surrounded by a transparent liquid called nucleoplasm. As cell division proceeds, the chromatin undergoes considerable coiling to form visible threadlike duplicate chromosomes that condense further to appear as 2 rodlike sister chromatids that are connected near the center at their centromeres. The microtubules of the *spindle apparatus* become visible and are involved in the separation on the sister chromatids. As the chromosomes complete their separation, vesicles begin to aggregate at the equator to form a cell plate (plants only) that eventually builds into a cell wall between the adjacent daughter cells.

Figure 6.2 Mitosis in plant cells. Note the square shape of the cells. In telophase, a cell plate develops and marks the boundary of the 2 daughter cells. (Courtesy Kendall/Hunt Publishing Company; © 1975 by Kendall/Hunt Publishing Company.)

Interphase | Early Prophase | Metaphase

Anaphase | Early Telophase | Daughter Cells

In figure 6.2 what is the diploid (2N) number of chromosomes in the nucleus of each cell? _____ (Counting the number of centromeres gives the chromosome number for the cell. The process of mitosis results in each daughter cell receiving duplicate copies of each kind of chromosome.)

Cell Cycle

There are 3 distinct phases of the *cell cycle: interphase*— nucleus appears normal; *mitosis*—the nuclear membrane disappears and the condensed chromosomes become visible and move apart; and *cytokinesis*— cytoplasmic cleavage. Upon completion, the cell returns to interphase.

During *interphase,* the nucleus appears normal and the cell is performing its cellular functions. The time spent in this phase varies from a few hours, in rapidly dividing cells (i.e., rapidly growing root tip), to the entire life of the mature individual (muscle cells). In cells that are actively dividing, *replication* (making of an exact copy of the DNA) occurs unseen toward the end of interphase. The DNA formed by replication forms 2 identical chromatids similar to those shown in figure 6.1 during the process of mitosis.

Observation of Mitosis Stages in a Plant Cell Cycle

In plants, the root tip contains tissue that is forever dividing and producing new cells.

1. Examine the prepared slide of *Allium* (onion) root tip cells undergoing mitotic cell division (fig. 6.3).
2. Using your labeled figure 6.2 and the descriptions following as aids, find one example of each stage of mitosis and the structures associated with mitosis.

Mitosis Model(s)

If mitosis models are available, examine them and fill in the following information.

Table 6.1 Mitosis Models

Identification			
Manufacture/Model no.			
Type (animal or plant)			
Diploid no.			

Interphase

The cell cycle consists of interphase plus the stages of mitosis. It is called a cycle because once mitosis occurs, interphase recurs. During interphase, replication of chromatin material (DNA) occurs. The nucleus appears similar to that in figure 6.1.

Mitosis

Prophase

The nuclear envelope is in the process of disappearing as a *spindle apparatus* appears. Distinct chromosomes, which are now visible, are randomly grouped in the center of the cell.

Metaphase

The nucleolus can no longer be seen. A fully formed spindle is composed of fibers that stretch from pole to pole. Each chromosome is attached to a spindle fiber at the centromere, and all the chromosomes are lined up at the *equator.*

Anaphase

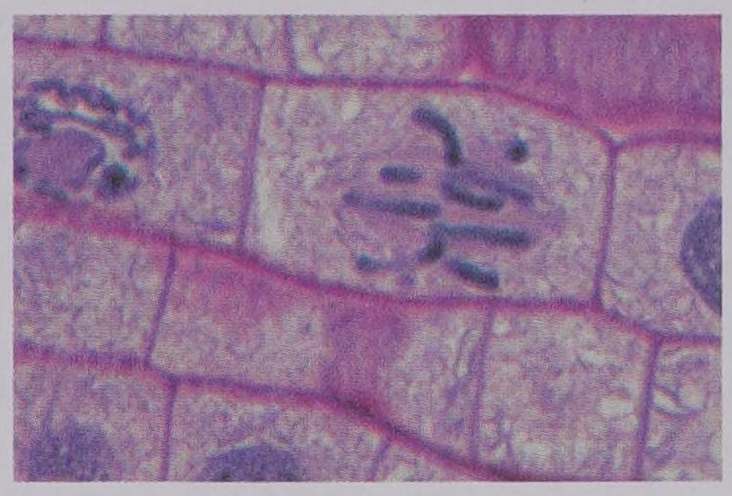
Centromeres divide; the chromatids *separate* and a set moves toward each pole.

Telophase

The chromosomes have clustered together near the spindle poles, where new nuclear envelopes develop around them. The spindle begins to disappear as the daughter nuclei form.

Figure 6.3 Photomicrographs of the stages of mitosis in an onion root tip.

Prophase



Metaphase

Anaphase

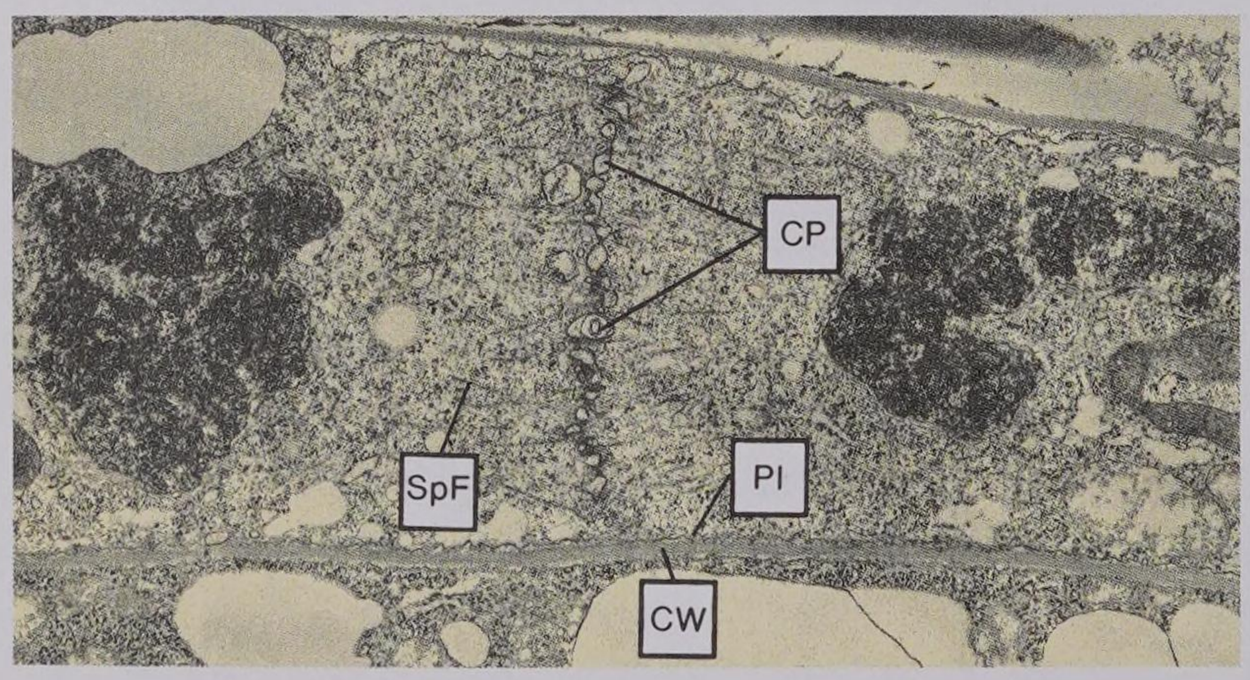
Telophase

Daughter Cells

Cytokinesis

Following mitosis, the cytoplasm divides by *cytokinesis*. In plant cells, membrane vesicles derived from the Golgi apparatus migrate to the center of the cell and form a *cell plate* (figs. 6.2, 6.4, and 6.5), the location of a new plasma membrane for each daughter cell. Later, individual cell walls will appear in this area.

Figure 6.4 Electron micrograph of a plant cell, showing cell plate formation during cytokinesis. Nuclear membranes have reformed. CP = cell plate; CM = cell membrane; CW = cell wall; Spf = spindle fibers.

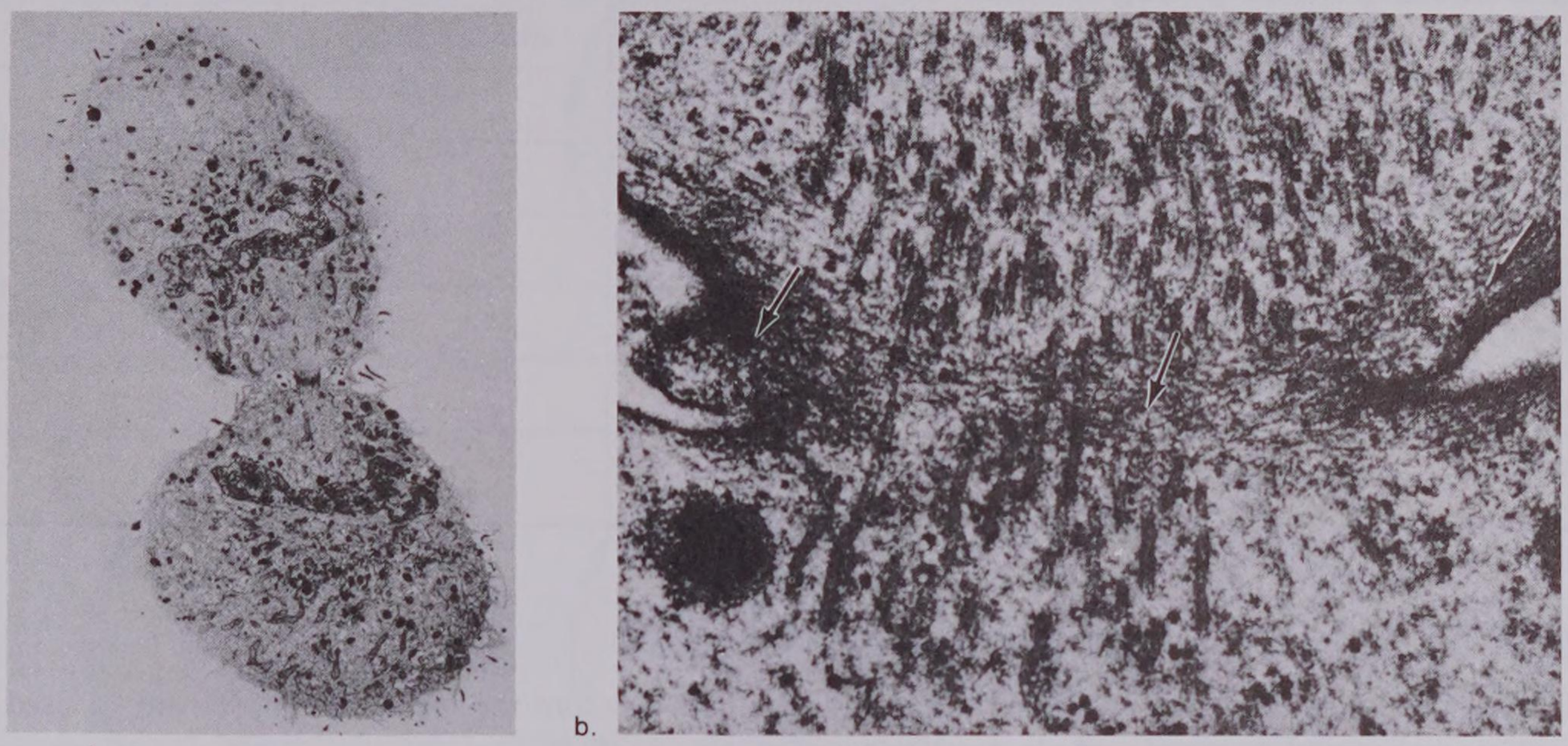


Summary

Summarize mitosis: The nucleus in the mother cell has the diploid (2N) number of chromosomes, and the nuclei in the daughter cells also have the _____ number of chromosomes.

Mitosis is nuclear division in which the chromosome number _____ .

Figure 6.5 ***a.*** Electron micrograph of human tissue culture cell in advanced stage of cytokinesis. Note the remnants of spindle fibers in furrow region. ***b.*** High magnification of a furrow region in a dividing rat cell. Arrows point to microfilaments. (***a:*** Courtesy of G. G. Maul and Academic Press, Inc. from *J. Ultrastr. Res.* 31:375 (1970). ***b:*** Courtesy of D. Syollosia, from *Journal of Cellular Biology* 44:192 (1970), by permission of the Rockefeller University Press.)



a. b.

Duration of Mitotic Stages (Optional)

One basic assumption of this exercise is that the more cells there are in a particular stage, the longer is the duration of that stage. A second assumption is that the duration from start to finish (including all 5 stages) is 24 hours (1440 min.).

S 1. Use the *Allium* root tip slide from the preceding exercise. Scan the root tip slide using your low power or scanning objective lens, and locate a region just behind the rounded tip that contains a high number of cells undergoing mitosis. These cells have near equal dimensions (not elongated). Change to the high power (45×) objective lens and select the field to be counted. Once a field has been chosen, the slide should not be moved, and only the cells within that field should be examined.

S 2. In this field, count the total number of cells and the number of nuclei in prophase, metaphase, anaphase, and telophase. Record these numbers in table 6.2.

S 3. *Total* the number of nuclei that were in prophase, metaphase, anaphase and telophase. These are mitotic figures. *Subtract* the number of mitotic figures from the total number of cells visible in this field. This gives you the number of cells in *interphase*. Record this number in the table 6.2.

Total mitotic figures = _____

Total number of cells − total mitotic figures = _____ nuclei in interphase

S 4. The duration of each mitotic stage may now be estimated using a calculator and the following equation:

$$\text{duration of mitotic stage} = \frac{\text{number of cells in a stage}}{\text{total number of cells}} \times 24 \text{ hr} \times \frac{60 \text{ min}}{1 \text{ hr}} =$$

5. Your instructor will pool the information for the class and give you the data to complete the second half of the table. Compare your personal data and class data to the reported data in the literature for the mitotic figures—25° C: prophase (42 min); metaphase (12 min); anaphase (6 min); telophase (30 min); and for interphase (1,350 min). Which is closer to the data in the literature, your data or those of the

class? _____

Can you suggest a reason for this? _____

_____

Table 6.2 Duration of Mitotic Stages

		Student		Class	
		Number of Cells	*Duration (min)*	*Number of Cells*	*Duration (min)*
	1. Total number of nuclei		———		———
Mitotic Figures	2. Number in prophase				
	3. Number in metaphase				
	4. Number in anaphase				
	5. Number in telophase				
	6. Number in interphase				

Observation of Mitosis Stages in an Animal Cell

The *blastula* is an early embryonic stage in the development of an animal from the fertilized egg. It will be used to emphasize the differences between animal and plant cell mitosis.

1. Look at figures 6.6 and 6.7 and identify the following distinctive structures and events of animal mitosis. You may note that during prophase in animal cells, the centrioles divide and the spindle arises between the 2 pairs of centrioles. By metaphase, each pair of centrioles has migrated to an opposite end of the cell, and the spindle fibers range between them. Each pair of centrioles is at the center of radially arranged short fibers called an *aster*. Centrioles are believed to give rise to the basal body present at the base of each flagellum and cilium.
2. Obtain a prepared slide of a whitefish blastula (whitefish mitosis), and examine it first on low power to find some cells with visible mitotic figures.
3. Switch to high power and find a cell or cells with all of the animal cell mitotic features listed previously.
4. Sketch in the space provided and label each of the features.

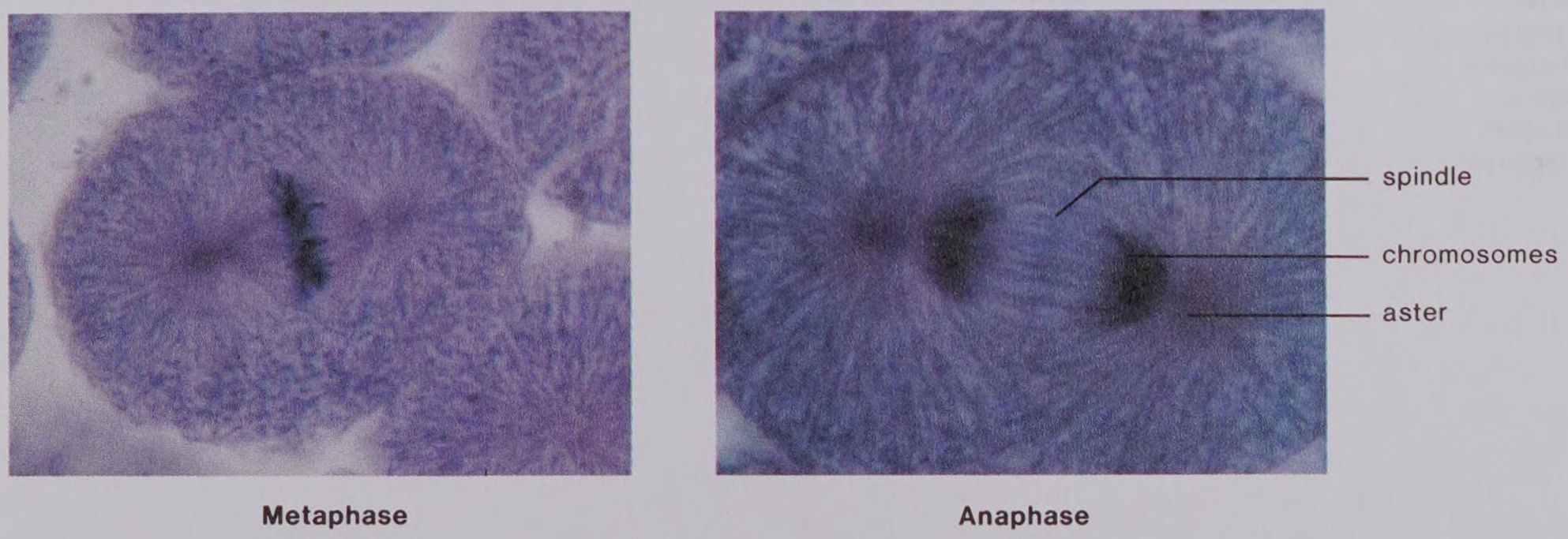
Cytokinesis

In animal cell cytokinesis, the cytoplasm divides by constricting inward in a process called *furrowing* (fig. 6.5*a*). Microfilaments in the area seem to take part in constricting the mother cell to produce the daughter cells.

Scan your slide to find a cell that is furrowing, add a sketch of this cell next to the others in the space provided, and *label*.

Figure 6.6 Mitosis in animal cells. Note the presence of an aster. (Courtesy of Kendall/Hunt Publishing Company; Copyright © 1975 by Kendall/Hunt Publishing Company.)

Figure 6.7 Whitefish blastula slides.

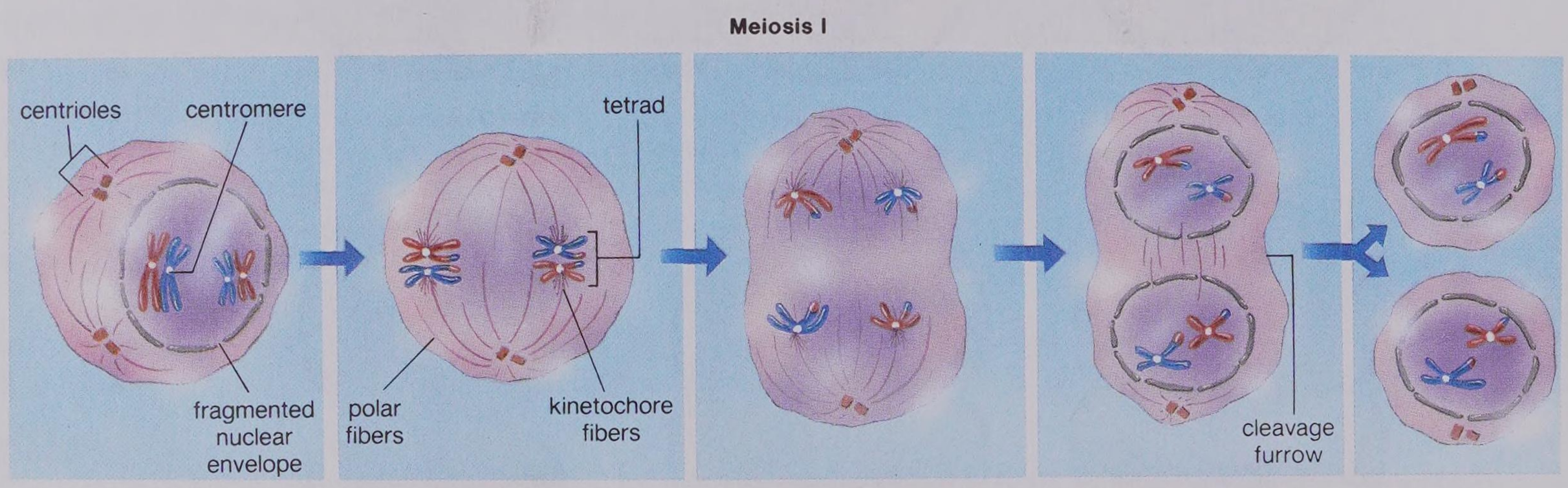


Meiotic Cell Division

Meiotic cell division is involved in egg and sperm production in animals and in spore production in fungi and plants. *Meiosis* is a form of nuclear division in which the chromosome number is reduced by one-half. A diploid (2N) nucleus contains the full number of chromosomes, and a haploid (N) nucleus contains half as many. While the nucleus of the mother cell has the diploid number of chromosomes, the daughter nuclei, after meiosis is complete, have the haploid number of chromosomes. In sexually reproducing species, meiosis must occur or the chromosome number would double with each generation.

Exactly the same stages of nuclear division are present as in mitosis; however, these stages occur twice—during *meiosis I* and *meiosis II*. In a diploid nucleus there are *homologous* chromosomes, chromosomes that look alike and carry genes for the same traits. During meiosis I, homologous chromosomes that are already double-stranded with sister chromatids (diads) (fig. 6.8) undergo *synapsis* (line up next to one another) and form *bivalents* (tetrads) before the homologous chromosome of each pair separate. During meiosis II, the sister chromatids of each chromosome separate. Because meiosis has 2 divisions, 4 nuclei result.

Figure 6.8 Meiosis consists of 2 divisions, meiosis I and meiosis II, and each of these has 4 stages. Members of homologous pairs have different colors to indicate that one member of each pair is a maternal chromosome and the other is a paternal chromosome. During meiosis I, the homologous chromosomes separate, and during meiosis II, sister chromatids separate.



Prophase I
Replication of DNA has occurred, and the chromosomes are duplicated. The homologous chromosomes pair, and while they are in synapsis, crossing-over between nonsister chromatids occurs. The chromosomes are condensing as prophase continues. Spindle fibers appear as the centrioles move apart and the nuclear envelope begins to fragment. Prophase I is the longest stage of meiosis and takes up most of the time required for the process.

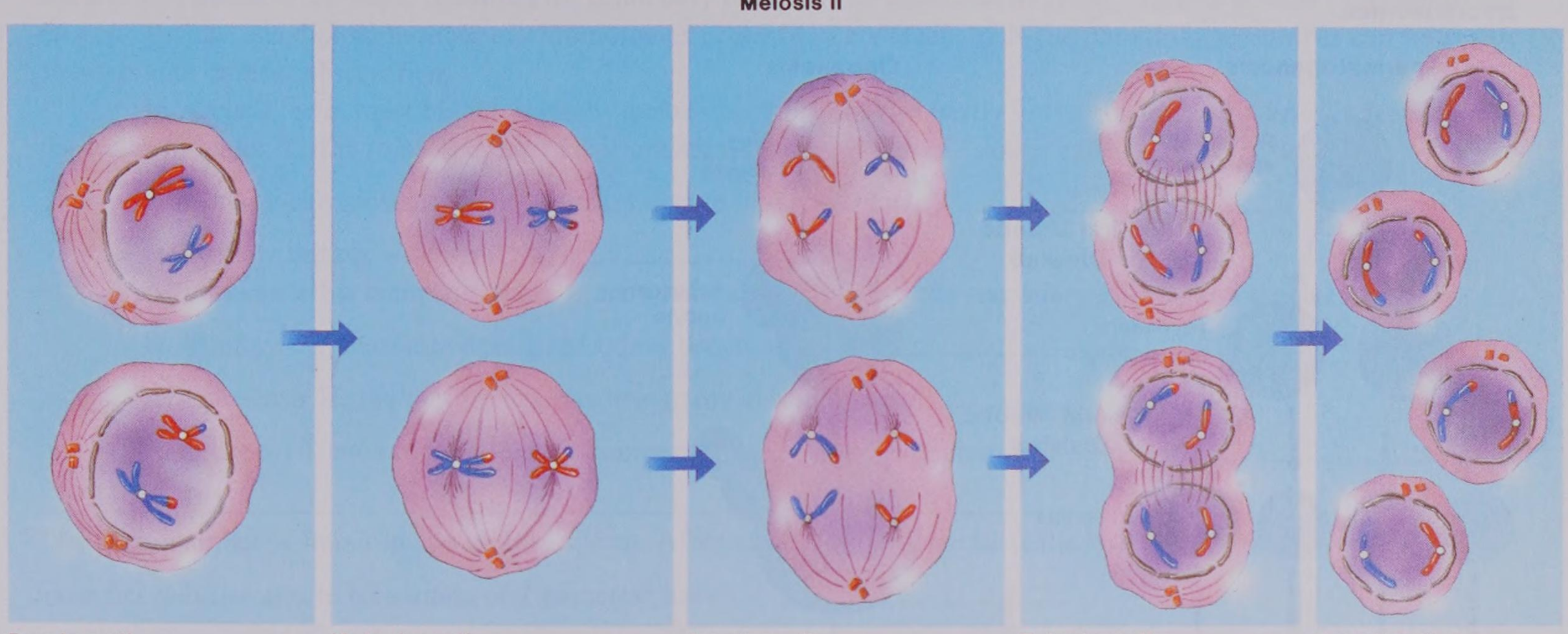
Metaphase I
The spindle apparatus is now fully formed and occupying the center of the cell. Homologue pairs (bivalents) are at the equator of the spindle. The kinetochores of the homologues point to opposite poles. Alignment of the homologues at the equator is completely random.

Anaphase I
The centrioles of sister chromatids remain intact, and the homologous chromosomes separate. Like mitosis, movement is due to a push provided by spindle elongation and a pull due to kinetochore disassembly.

Telophase I
The haploid number of chromosomes is at each pole. Assortment is independent and all possible combinations of chromosomes may occur at the poles. This figure shows only 2 possible combinations—what are the other possible combinations? Usually cytokinesis occurs simultaneously with telophase.

Daughter Cells

Figure 6.8 Continued



Prophase II
No replication of the DNA occurs between meiosis I and meiosis II—the chromosomes were duplicated earlier. Condensation of chromosomes begins once again. The spindle forms and the nuclear envelope fragments once more.

Metaphase II
The duplicated and fully condensed chromosomes are aligned individually at the equator. The kinetochore fibers of the sister chromatids point in opposite directions. This is in preparation for their separation.

Anaphase II
Sister chromatids separate just as they do during mitosis, and that's why it is said that the events of meiosis II are like those of a mitotic division. The chromatids, now called chromosomes, move toward opposite poles.

Telophase II
Each daughter nucleus contains the haploid number of chromosomes, and these chromosomes are single. The daughter nuclei are not genetically similar to the mother cell. They have the haploid number of chromosomes, and due to crossing-over during prophase I, these chromosomes may carry a new rearrangement of the genetic material.

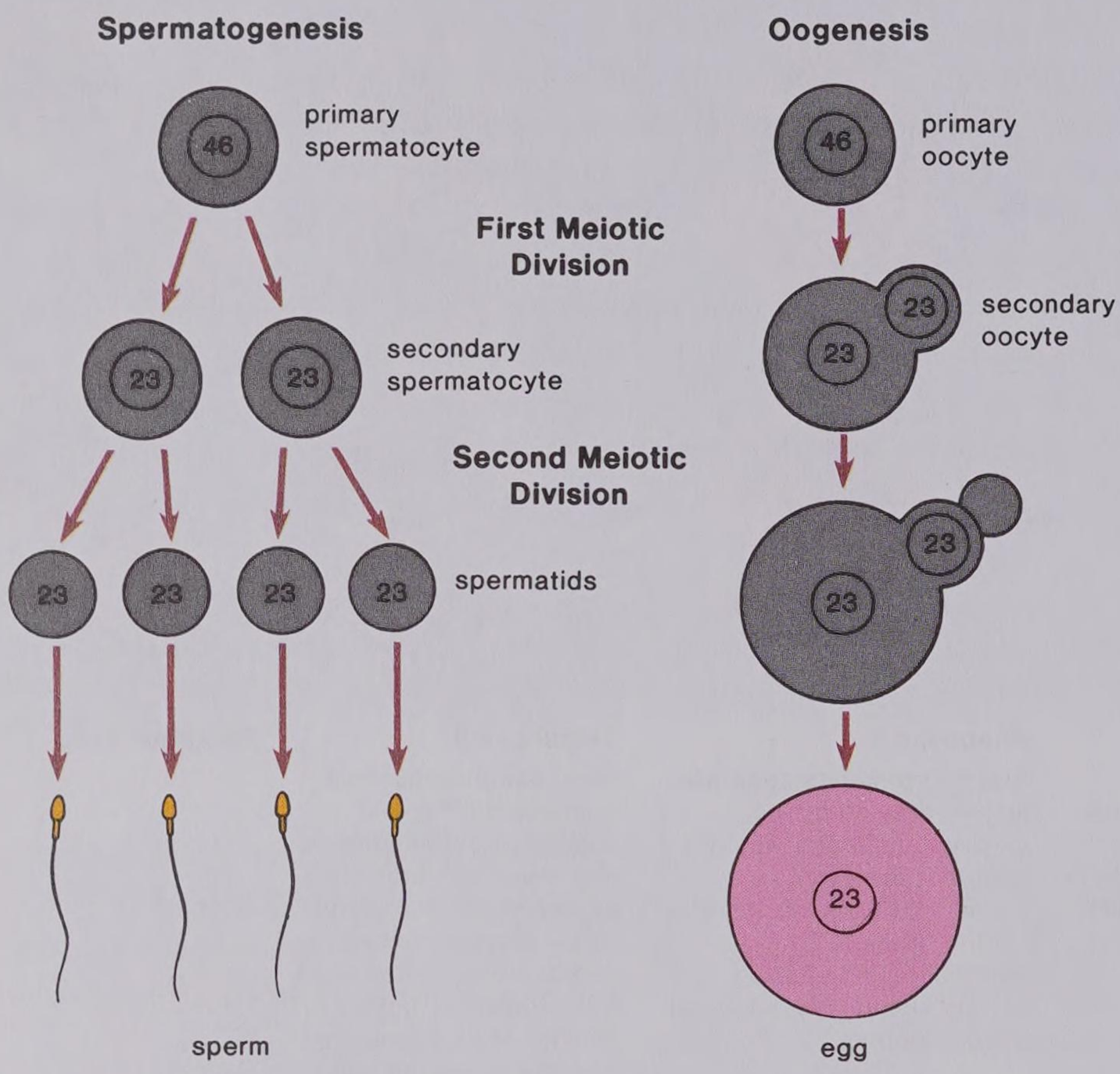
Daughter Cells

Stages of Meiosis

1. Examine figure 6.8 and identify the structures and processes described previously. Next to each cell, write 2N or N.
2. A count of the number of centromeres tells how many chromosomes a nucleus has.
3. Identify on figure 6.1: metaphase I, anaphase I, anaphase II, and telophase II. *Label* the chromosomal configuration tetrad (bivalent) and label a diad (sister chromatids) and a monad (one chromatid).

The general term for meiosis, which forms *gametes* (sex cells), is *gametogenesis*. Gametogenesis that produces the male gamete (sex cell), sperm in animals and pollen grains in plants, produces 4 haploid (N) cells as illustrated in figure 6.8. In male animals, this process produces sperm and is called *spermatogenesis* (fig. 6.9). In females, gametogenesis produces eggs and is called *oogenesis*. It results in the production of one functional egg and 2 nonfunctional cells called *polar bodies*.

Figure 6.9 Spermatogenesis produces 4 viable sperm, whereas oogenesis produces one egg and at least 2 polar bodies. In humans, sperm and egg have 23 chromosomes each; therefore, following fertilization, the zygote has 46 chromosomes.



Meiosis Models

If meiosis models are available, examine them and place the information in table 6.3.

Table 6.3 Meiosis Models

	General	Spermatogenesis	Oogenesis
Manufacturer			
Model no.			
Diploid no.			
Haploid no.			
No. viable cells produced			

Reminder: To count chromosome numbers count the number of centromeres visible in the cell.

Summary

Summarize meiosis: The nucleus in the mother cell has the diploid (2N) number of chromosomes, and the nuclei in the daughter cells have the haploid (N) number of chromosomes. Meiosis is nuclear division in which the chromosome number

_____

_____ .

Meiosis in Ascaris

Ascaris is a genus of parasitic roundworms commonly found in the intestines of swine. *Ascaris* is used to demonstrate meiosis because the diploid number of chromosomes is only 4. This means that the chromosome number can be easily counted with careful observation.

In *Ascaris,* as in most higher animals, meiosis occurs wholly or partly in the gonads, the ovaries in females and the testes in males. In the female, a cell that is undergoing meiotic cell division is called a *primary oocyte.*

How many chromosomes will a primary oocyte in *Ascaris* have? _____

How many tetrads will form? _____

After meiosis I is complete, there is one functional cell, called the *secondary oocyte,* and a polar body.

How many chromosomes does a secondary oocyte have? _____

After meiosis II, the mature egg has how many chromosomes? _____

In figure 6.10, each of these chromosomes is called a *monad.* Explain this term. _____

_____

The same number is found in the mature sperm. After egg and sperm nuclei (called *pronuclei*) fuse, how many chromosomes will the zygote (the union of 2 gametes) have? _____

Ascaris Slide

S 1. Obtain a slide containing a longitudinal section of the oviduct and uterus of *Ascaris.* The oviduct can be distinguished from the uterus because it has a smaller diameter. As you examine the slide under low power, you will see a mixture of round, maturing eggs and triangular sperm. *Oogenesis began in the ovaries but continues as the primary oocyte travels down the oviduct after a sperm has entered.* Once a sperm has entered a primary oocyte, the primary oocyte starts to secrete a shell, while the sperm pronucleus (termed *pronucleus* because fusion with the egg nucleus has not yet occurred) rests.

S 2. Switch to high power, and identify as many stages of oogenesis as possible, using figure 6.10 as a guide. List the stages that you observed.

_____

Mitosis versus Meiosis

S Examine figure 6.11, and note differences between mitosis and meiosis. Fill in table 6.4 to indicate these differences.

Table 6.4 Mitosis and Meiosis

	Mitosis	Meiosis
1. Number of divisions		
2. Chromosome number of daughter cells		
3. Number of daughter cells		
4. Produces gametes		

Figure 6.10 Diagram of the female reproductive tract of *Ascaris,* showing distribution of meiotic and developmental egg stages.

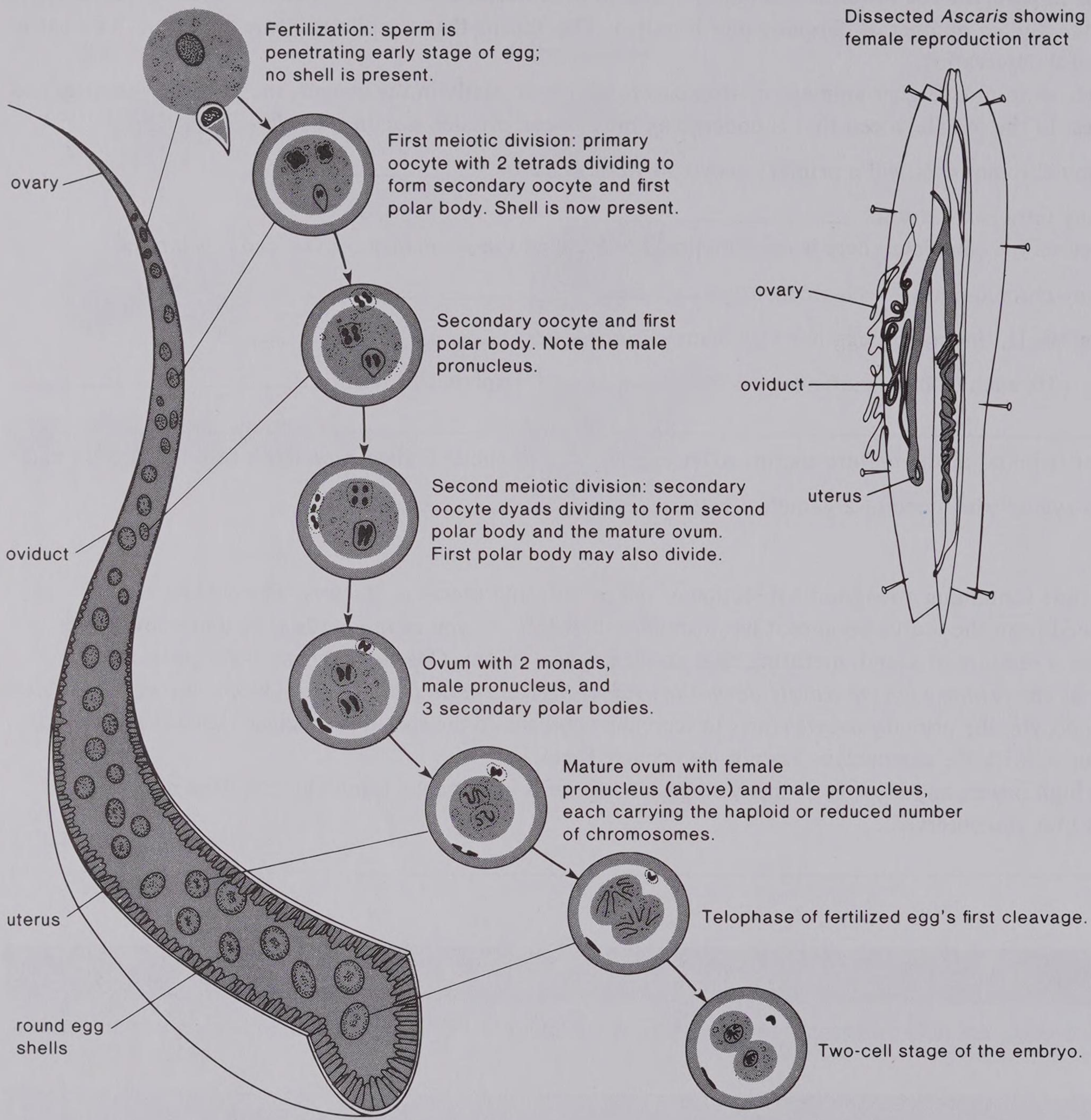
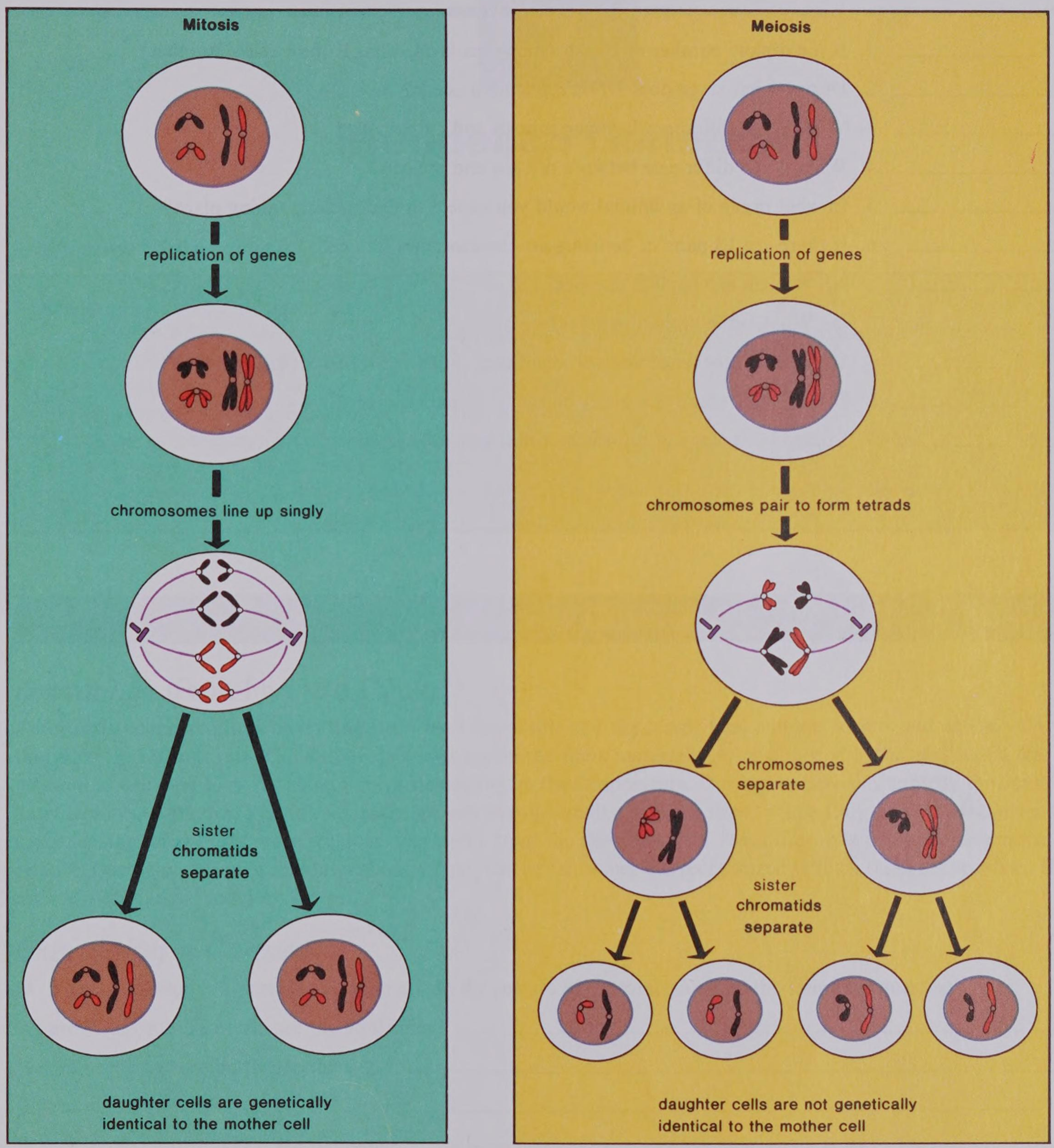

Fertilization: sperm is penetrating early stage of egg; no shell is present.
First meiotic division: primary oocyte with 2 tetrads dividing to form secondary oocyte and first polar body. Shell is now present.
Secondary oocyte and first polar body. Note the male pronucleus.
Second meiotic division: secondary oocyte dyads dividing to form second polar body and the mature ovum. First polar body may also divide.
Ovum with 2 monads, male pronucleus, and 3 secondary polar bodies.
Mature ovum with female pronucleus (above) and male pronucleus, each carrying the haploid or reduced number of chromosomes.
Telophase of fertilized egg's first cleavage.
Two-cell stage of the embryo.
ovary
oviduct
uterus
round egg shells

Figure 6.11 Mitosis versus meiosis.


Mitosis
replication of genes
chromosomes line up singly
sister chromatids separate
daughter cells are genetically identical to the mother cell
Meiosis
replication of genes
chromosomes pair to form tetrads
chromosomes separate
sister chromatids separate
daughter cells are not genetically identical to the mother cell

Laboratory Review 6

_____ 1. If the diploid number of the chromosomes is 54, what is the haploid number?

_____ 2. During what stage does DNA replication occur?

_____ 3. What is the difference between mitosis and cytokinesis?

_____ 4. What is the difference between mitosis and meiosis?

_____ 5. In what organ of an animal would you expect to find meiosis taking place?

_____ 6. If there are 13 pairs of homologous chromosomes in a cell:

_____ a. What is the haploid number?

_____ b. What is the diploid number?

_____ 7. Why is the process of meiosis sometimes called reduction division?

_____ 8. During what stage of meiosis do the bivalents separate?

_____ 9. During what stage of mitosis do the chromatids separate?

References

Department of Biology, Nassau Community College. 1973. *General Biology I.* Dubuque, IA: Kendall/Hunt Publishing Company.

Longenecker, Nevin E. 1981. A simplified method for observing mitosis. *American Biology Teacher* 43(3):160.

Sunberg, Marshall D. 1981. Making the most of onion root tip mitosis. *American Biology Teacher* 43(7):386.

Laboratory

7

Mendelian Genetics

Learning Objectives

Students should be able to

1. describe the life cycle of *Drosophila* (Fruit Fly) and recognize these stages in a culture bottle;
2. distinguish male flies from female flies;
3. recognize the 3 types of mutants utilized for crosses;
4. give the phenotype and genotype for all flies mentioned;
5. describe and explain the schedule for the assigned cross;
6. predict the results of all crosses for both the F_1 and F_2;
7. utilize the chi-square statistical test to help determine whether data does or does not support a hypothesis.

Drosophila Cultures

Drosophila (Fruit Fly) Life Cycle

Drosophila occur naturally on rotting fruit; both the adults and the larvae feed on plant sugars and on the wild yeasts that grow on rotting fruit. The female flies lay eggs on the same materials. After a day or 2, the eggs hatch into small larvae that feed and grow for about 8 days, depending on the temperature. During the period of growth, they molt twice and there are, therefore, 3 larval periods of growth between molts, called *instars*. When fully grown, third instar larvae cease feeding, crawl to a more secluded spot away from the decaying fruit (onto the walls of the culture vessel), and *pupate*. During pupation, which lasts about 4 days, the larval tissues are reorganized to form those of the adult. The life cycle is summarized in figure 7.1.

Examining a Culture

Your instructor will provide you with a fruit fly culture to examine. Answer the following questions.

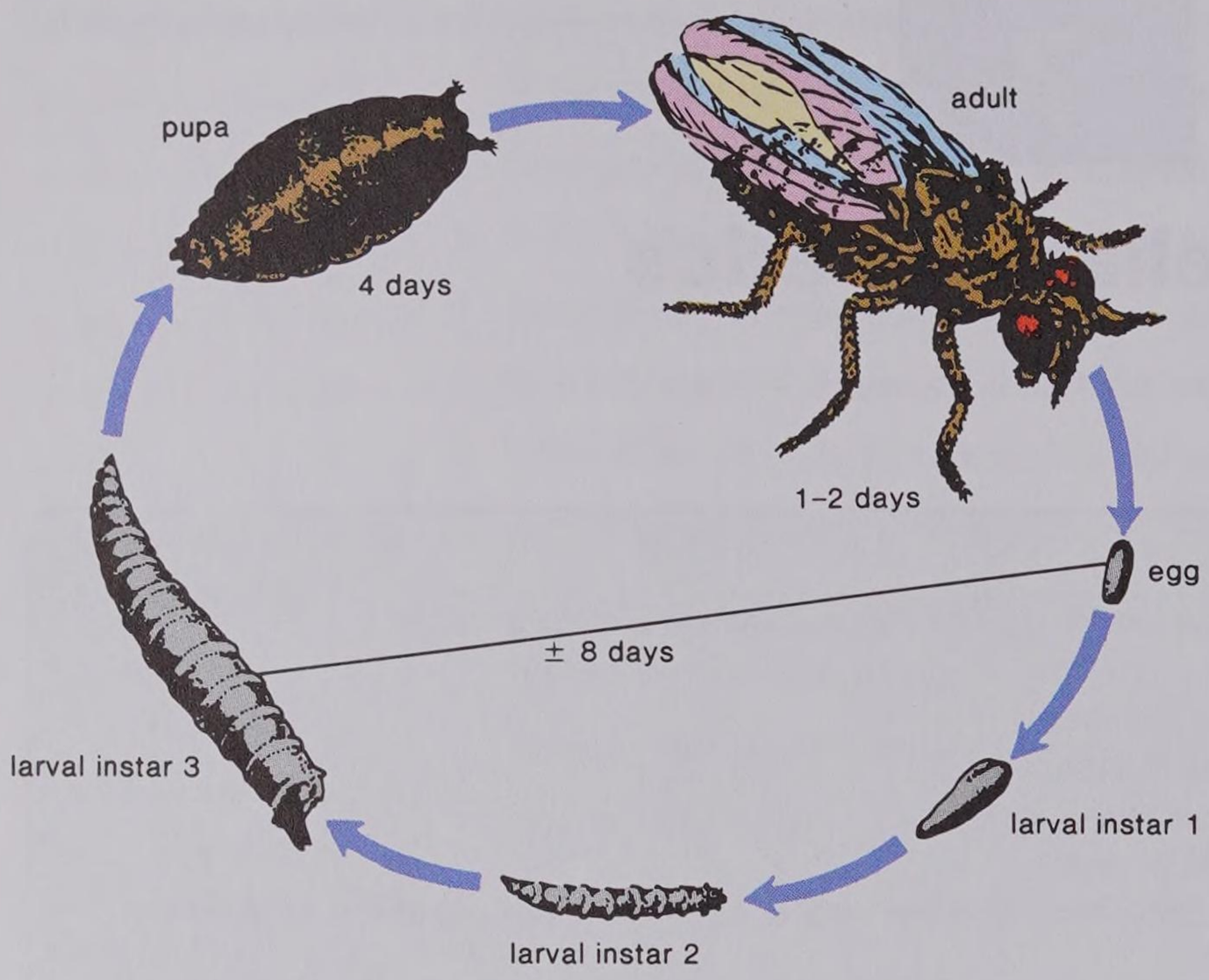
Where in the culture bottle are the adult flies? _____

Where in the culture bottle are the eggs? _____

Where in the culture bottle are the larvae? _____

Where in the culture bottle are the pupae? _____

Figure 7.1 Life cycle of *Drosophila*.



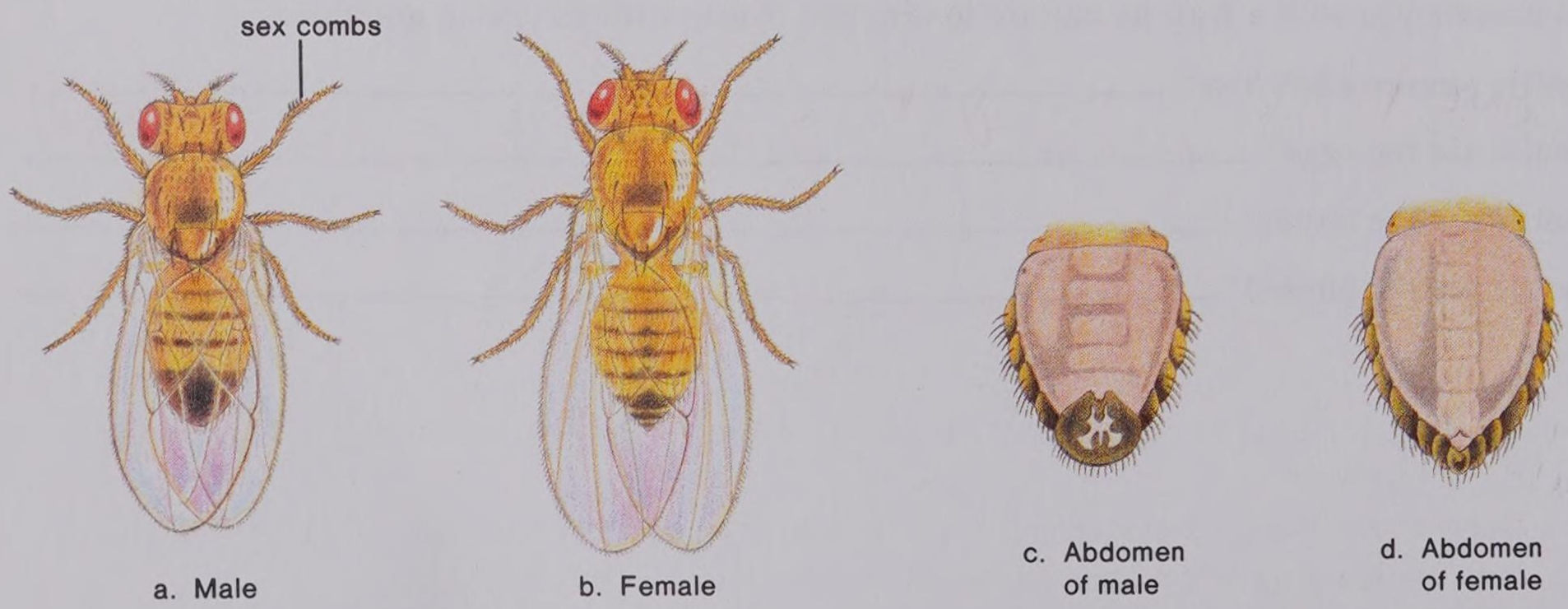
Adult Flies

Male and Female Characteristics

To distinguish male from female, the following characteristics may be helpful (fig. 7.2).

1. The male is generally smaller.
2. Dorsally, the male is seen to have a black-tipped abdomen, while the female appears to have dark lines only at the tip.
3. Ventrally, the abdomen of the male has a dark region at the tip, a feature lacking in the females.
4. The male has a more rounded abdomen than the female. The female has a pointed abdomen.
5. The male has a sex comb on the forelegs.

Figure 7.2 *Drosophila* characteristics.



Phenotype and Genotype

The outward appearance of organisms is called their *phenotype*. The actual genetic makeup, called the *genotype*, can be determined only by successive crosses and observation of phenotypes.

In regard to the flies you are examining today, we can tell the phenotype by looking at them; we know the genotype because the supply house has carried out the required successive crosses.

Wild-Type

The wild-type fly has the phenotype we associate with the normal fruit fly: *red eye, long wings,* and *gray body*. To indicate the genotype, it is necessary to know that there are 2 *alleles* (alternative forms of the gene) for every trait. The traits we are dealing with are eye color, wing length, and body color. Also, it must be known that the wild-type characteristics are dominant and, therefore, are designated by a capital letter. Notice the phenotype and genotype that have been noted for the wild-type fly in table 7.1. It is necessary to indicate the eye-color alleles as superscripts of the chromosome X because these alleles are on this particular sex chromosome. Your instructor will explain more about *X-linked genes* at this laboratory or the next one.

Mutants

We will work with 3 mutant types: *white eye, vestigial* (*short*) *wings,* and *ebony body*. Notice that each mutant differs from the wild-type in only one phenotype characteristic. This means that its genotype will differ only in regard to alleles for this characteristic. Knowing that the mutant characteristics are recessive and designated by a lowercase letter, ❶ *examine* the flies that are available and enter the data in table 7.1. (If your instructor directs you to anesthetize the flies first read the following section.)

Table 7.1 Wild and Mutant Flies

Trait	Wild-type	White Eye	Vestigial	Ebony
Eyes: *Phenotype*	red eye			
Genotype	X^RX^R, X^RY			
Wing length: *Phenotype*	long wings			
Genotype	*LL*			
Body: *Phenotype*	gray body			
Genotype	*GG*			

Observation of Flies

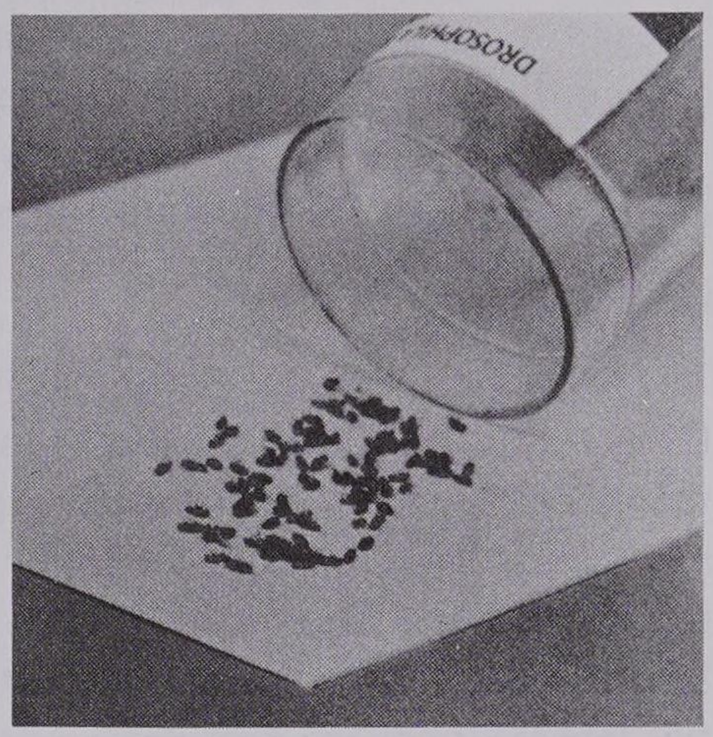
Anesthetizing Flies

The use of FlyNap[1] allows flies to be anesthetized for at least 50 minutes without killing them.

❶ 1. Dip the absorbent end of the wand into the FlyNap bottle as shown in figure 7.3.
❶ 2. Tap the bottom of the culture vial on the tabletop to knock the flies to the bottom of the vial. With one finger, push the plug slightly to one side.
❶ 3. Remove the wand from the FlyNap and quickly stick the anesthetic end into the culture vial beside the plug so that anesthetic tip is below the plug. Keep the culture vial upright with the wand in place.
❶ 4. Remove the plug and wand immediately after the flies are anesthetized (approx. 2 min in an empty vial, 4 min in a vial with medium), and spill them out onto a white file card.

[1]FlyNap is a registered trademark of Carolina Biological Supply Company.

Figure 7.3 Use of FlyNap.



5. When replugging the culture vial, be careful not to insert a part of the plug that may have FlyNap on it.
6. The length of time the flies remain anesthetized depends on the amount of FlyNap on the wand and on the number and age of the flies in the culture vial.
7. If the medium is not firm, you may wish to transfer flies to an empty bottle before anesthetizing them. (Ask your instructor for assistance if this is the case.)
8. Put the anesthetized flies, which are on a white card, onto the glass plate of a binocular dissecting microscope for examination, or use a hand lens.
9. Use a camel hair brush to move flies. Separate male and female wild-type flies. Have your instructor check to see if you have separated them correctly.
10. Examine the mutant flies carefully to be sure you can identify the wing length, eye color, and body color for both normal and mutant flies.
11. Return the flies to the culture bottle or dispose of them in a "morgue" as directed by your instructor.

Experimental *Drosophila* Crosses

You will choose or be assigned one of the following crosses listed. The phenotypes given are those of the *P* (*parent flies*). The supply house has guaranteed the flies are *true-breeding*. True breeding means that the organism is *homozygous*, dominant or recessive, and their offspring will also be homozygous. The offspring from these parent flies are called the F_1 (first filial) generation. The offspring from the F_1 flies are called the F_2 (second filial) generation. F_2 flies represent your final results; these flies must be examined closely and counted.

The following individuals are homozygous and possess a pair of identical genes for that specific trait. All of the other genes they possess are wild-type genes for which they are also homozygous. The trait mentioned in parentheses is the wild-type condition for that trait. (Heterozygous individuals have two different kinds of genes for a specific trait.)

Suggested Crosses (♂ = males; ♀ = females)

1. Monohybrid—Simple (Autosomal, non–X-linked)
   a. wild-type (long wing) ♂ × vestigial wing ♀
   b. wild-type (long wing) ♀ × vestigial wing ♂
   c. wild-type (grey body) ♂ × ebony body ♀
   d. wild-type (grey body) ♀ × ebony body ♂
2. Monohybrid—Simple (X-linked)
   e. white eye ♂ × wild-type (red-eyed) ♀
   f. white eye ♀ × wild-type (red-eyed) ♂
3. Dihybrid—Complex (Autosomal, non–X-linked)
   g. ebony body, (long wing) ♂ × (grey body), vestigial wing ♀
   h. ebony body, (long wing) ♀ × (grey body), vestigial wing ♂

4. Dihybrid—Complex (X-linked)
   i. white eye, (long wing) ♂ × (red eyed), vestigial wing ♀
   j. white eye, (long wing) ♀ × (red eyed), vestigial wing ♂

Five-Week Experiment

- (T) If you are given P flies, your experiment will take 5 weeks.
- (S) Week 1: Place the parent flies in a culture bottle that contains Instant Drosophila Medium.[2] (Equal volumes of formula 4–24 Instant Drosophila Medium and cool tap water are dumped into the vial and a very few grains [4–6 grains] of dry yeast are sprinkled on top.) Record your cross in the results section.
- (S) Week 2: Remove the parent flies from the culture bottle in order not to confuse them with the offspring that will soon hatch out.
- (S) Week 3: Observe the F_1 generation and record your results. Transfer some of these F_1 flies to a new culture bottle.
- (S) Week 4: Remove the F_1 flies from the bottle before the F_2 flies hatch.
- (S) Week 5: Count the F_2 generation and complete the record of your data.

F_1 flies are the first generation to appear in about 2 weeks after parent flies are placed in the culture bottle. Why is it important to remove parent flies from the culture bottle before F_1 flies hatch? _____

_____

F_1 flies continue to hatch for several weeks after their first appearance. Why should F_1 flies be placed in a new culture bottle at the third week? _____

_____

Why should the F_1 flies be removed before the F_2 flies appear? _____

_____

Describe the method you will use to count your F_2 flies. _____

_____

_____

Three-Week Experiment

- (T) If you are given F_1 flies, your experiment will take 3 weeks. Fill in the first half of the results section just as if you had performed this portion of the experiment yourself. Examine the F_1 flies and fill in the appropriate information under First Filial Generation (F_1).
- (S) Week 1: Place the F_1 flies in a culture bottle, and complete the first half of the results section.
- (S) Week 2: Remove the F_1 flies from the bottle before the F_2 flies hatch out.
- (S) Week 3: Count the F_2 generation, and complete the record of your data.

Why should the F_1 flies be removed before the F_2 flies appear? _____

Describe the method you will use to count your F_2 flies. _____

_____

_____

Results

Parental Generation (P)

Phenotypes _____ × _____

Genotypes _____ × _____

[2]Formula 4–24 Instant Drosophila Medium is a registered trademark of Carolina Biological Supply Company.

First Filial Generation (F_1)

Give your actual results for the F_1 generation.

F_1 phenotypes _____ × _____

Genotypes (calculated) _____ × _____

Second Filial Generation (F_2)

Give your actual results in table 7.2 for the F_2 generation.

Table 7.2 F_2 Generation Results

F_2 Phenotypes	Number of Flies		
	♀ *(X-linked only)*	♂ *(X-linked only)*	*Total*
Total:			

Predicting Genetic Ratios—Punnett Square

One method of predicting the expected genetic ratios is the Punnett square method. There are additional methods that may be explained in your text or by your instructor. This explanation and example is only for a monohybrid cross.

The following cross is used as an example: two flies are heterozygous (one dominant, one recessive gene for a trait) for vestigial wing; their phenotype (visible expression of a gene) is the dominant wild-type allele (alternate gene form) long wing, and their genotype (genes for the trait) is *Ll*. The following symbols are used:

Genotype	**Phenotype**
LL	long wing (homozygous dominant)
Ll	long wing (heterozygous)
ll	vestigial wing (homozygous recessive)

(Reminder: Be sure to keep the same letter for different alleles of the same trait; use a capital letter for the dominant trait and a lowercase letter [or a series of ', '', ' ''] for the recessive trait.)

The gametes must be determined. For a homozygous individual, all of the gametes are the same (i.e., *LL* = all [100%] *L*). For heterozygous individuals, 1/2 are dominant and 1/2 are recessive (i.e., *Ll* = 1/2 *L*, 1/2 *l*). Once symbols are defined, the phenotypes, genotypes, and gametes are determined. The Punnett square can be set up with the gametes of one parent on one axis and the gametes of the other parent on the other axis. So for the cross:

Phenotype:	long wing ♂	×	long wing ♀	(both are heterozygous for vestigial wing)
Genotype:	*Ll*	×	*Ll*	

Gametes: 1/2 *L*, 1/2 *l*; 1/2 *L*, 1/2 *l*

♂ sperm \ ♀ eggs (gametes)	*L*	*l*
L	*LL* long wing (wild-type)	*Ll* long wing (wild-type)
l	*Ll* long wing (wild-type)	*ll* vestigial wing

Expected Ratios: Phenotypic—3/4 long wing; 1/4 vestigial
Genotypic—1/4 *LL*; 2/4 *Ll*; 1/4 *ll*

Expected Results

Predict your expected ratio for the F_1 generation and the F_2 generation using the Punnett square method.

F_1

F_2

Give the actual phenotype ratio you observed: _____

Compare the actual ratio with the expected ratio: _____

Are they the same? _____ exactly? _____

Can you explain (e.g., based on probability of results) why your results might not be exact? _____

_____

Chi-Square Analysis (Optional)

It is possible to evaluate your results by using the *chi-square* (χ^2) test. This is the statistical test most frequently used to determine whether data obtained experimentally provide a good fit, or approximation, to the expected or theoretical data. Basically, this test can be used to determine whether any deviations from the expected values are due to chance.

Chance alone can cause the actual observed ratio to vary somewhat from the calculated ratio for a genetic cross. For example, very rarely will a ratio of exactly 3:1 be observed for a monohybrid cross. The actual results will differ, but there comes a point when the difference is so great as to be unexpected. The chi-square test indicates this point. After this point, the original hypothesis (e.g., that a monohybrid cross gives a 3:1 ratio) is not supported by the data.

The formula for the test is as follows:

$$\chi^2 = \Sigma(d^2/e),$$

χ^2 = chi-square

Σ = sum of

d = difference between expected and observed results (most often termed the deviation)

e = expected results

Example

In the cross *wild-type* by *vestigial,* a 3:1 ratio is expected. Thus, if a student counts 160 flies, 40 are expected to have vestigial wings, while 120 are expected to have long wings, But if a student counts 44 vestigial-winged flies and 116 long-winged flies, then the value for the chi-square test would be as calculated as in table 7.3.

Table 7.3 Calculation of Chi-Square

Phenotype	Observed Number	Expected Number (*e*)	Difference (*d*)	d^2	Partial Chi-Square d^2/e
Vestigial wings	44	40	4	16	16/40 = .400
Long wings	116	120	4	16	16/120 = .133
					$\Sigma(d^2/e) = .533$
					$\chi^2 = .533$

Now it is necessary to look up this chi-square value (χ^2) in a table that indicates whether the probability (p) is that the differences noted are due only to chance in the form of random sampling error *or* whether it would be better to explain the results on the basis of a different prediction (hypothesis).

In table 7.4, the notation C refers to the number of "classes," which in this laboratory would be determined by the number of phenotypic traits studied. In our example we have 2 classes, vestigial wings and long wings; however, as indicated in the table by $C - 1$, it is necessary to subtract one from the total number of classes. Thus, since we had 2 classes, it is necessary to look for our χ^2 value in the first line of the table. The value, .533, lies between $\rho = .50$ and .30. This means that, by random chance, this difference between the actual count and the expected count would occur between 30% and 50% of the time. In biology, it is generally accepted that a ρ value greater than .05 is acceptable, while a ρ value lower than .05 would indicate that the results cannot be due to random sampling and therefore do not support (do not fit) the original prediction (hypothesis).

Table 7.4 Values of Chi-Square

	Hypothesis Is Supported							Hypothesis Is Not Supported		
	Differences Insignificant							*Differences Significant*		
ρ / C − 1	.99	.95	.80	.50	.30	.20	.10	.05	.02	.01
1	.00016	.0039	.064	.455	1.074	1.642	2.706	3.841	5.412	6.635
2	.0201	.103	.446	1.386	2.408	3.219	4.605	5.991	7.824	9.210
3	.115	.352	1.005	2.366	3.665	4.642	6.251	7.815	9.837	11.341
4	.297	.711	1.649	3.357	4.878	5.989	7.779	9.488	11.668	13.277
5	.554	1.145	2.343	4.351	6.064	7.289	9.236	11.070	13.388	15.086

Source: Data from Keeton, et al., *Laboratory Guide for Biological Science,* page 189, 1968.

Analysis

Do a chi-square analysis of your results by filling in table 7.5. If you performed a one-trait cross, you will require only the first 2 lines. If you performed a 2-trait cross, you will require 4 lines.

Table 7.5	Actual Results				
Phenotype	**Observed Number**	**Expected Number (*e*)**	**Difference (*d*)**	**d^2**	**Partial Chi-Square d^2/e**
					=
					=
					=
					=

$\Sigma(d^2/e)$ =

χ^2 = _____

C − 1 = _____

ρ (from table 7.4) = _____

Do your results support your original prediction? _____

If not, can you account for this? _____

_____

Summary of Expected Results

❸ Although you have been assigned only one particular cross, you are responsible for understanding all of them. Determine and record the expected results for all crosses.

One-Trait Crosses

A one-trait cross is one in which each individual has one allelic pair.

Key: G = grey body; g = ebony body
L = long wing; l = vestigial wing
R = red eye; r = white eye

1. *Monohybrid*—Simple (Autosomal, non-X-linked)

   a. wild-type (long wing) ♂ × vestigial wing ♀

   or

   b. wild-type (long wing) ♀ × vestigial wing ♂

P genotypes _____ × _____

F_1 phenotype _____

F_1 genotype _____

F_2 phenotype ratio: _____ : _____

   c. wild-type (grey body) ♂ × ebony body ♀

   or

   d. wild-type (grey body) ♀ × ebony body ♂

P genotypes _____ × _____

F_1 phenotype _____

F_1 genotype _____

F_2 phenotype ratio: _____ : _____

2. *Monohybrid*—Simple (X-linked)

e. white eye ♂ × wild-type (red eyed) ♀

P genotypes _____ × _____

F_1 phenotype _____ (male) × _____ (female)

F_1 genotype _____ (male) × _____ (female)

F_2 phenotype ratio: (male) _____ : _____ (female) _____ : _____

f. white eye ♀ × wild-type (red eyed) ♂

P genotypes _____ × _____

F_1 phenotype _____ (male) × _____ (female)

F_1 genotype _____ (male) × _____ (female)

F_2 phenotype ratio: (male) _____ : _____ (female) _____ : _____

3. *Dihybrid*—Complex (Autosomal, non-X-linked)

g. ebony body, (long wing) ♂ × (grey body), vestigial wing ♀

h. ebony body, (long wing) ♀ × (grey body), vestigial wing ♂

P genotypes _____ × _____

F_1 phenotype _____

F_1 genotype _____

F_2 phenotype ratio: _____ : _____ : _____ : _____

4. *Dihybrid*—Complex (X-linked)

i. white eye, (long wing) ♂ × (red eyed), vestigial wing ♀

P genotypes _____ × _____

F_1 phenotype _____ (male) × _____ (female)

F_1 genotype _____ (male) × _____ (female)

j. white eye, (long wing) ♀ × (red eyed), vestigial wing ♂

P genotypes _____ × _____

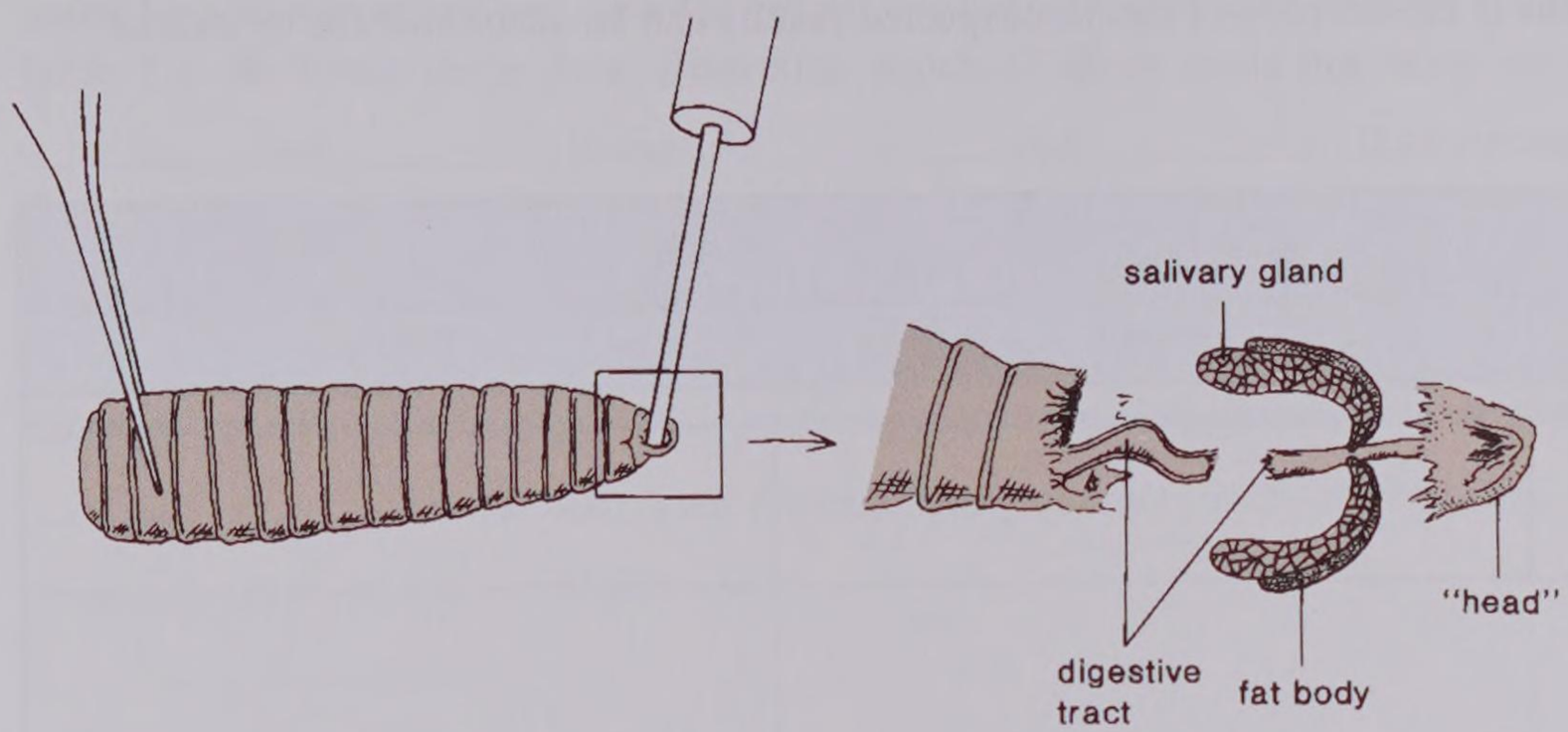
F_1 phenotype _____ (male) × _____ (female)

F_1 genotype _____ (male) × _____ (female)

Drosophila Chromosomes (Optional)

Genes are located on the chromosomes; therefore, geneticists are very much interested in the structure and function of chromosomes. While chromosomes are often difficult to see during interphase, this is not the case with the chromosomes found within the salivary glands of fully mature *Drosophila* larvae. These chromosomes are so large because (1) the chromosomes in each cell of the glands have duplicated and remain adhering to each other and (2) the homologous chromosomes are attached along their entire length.

Figure 7.4 Removing salivary glands from *Drosophila* larva.



To observe the chromosomes, follow these directions:

1. Take a larva that is about to pupate from a noncrowded culture bottle.
2. Transfer the larva from the culture to a clean slide wetted by a drop of *0.7% NaCl.*
3. Use a binocular dissecting microscope to locate the anterior end (pointed, with black mouthparts). Put a dissecting needle through the head as close to the mouthparts as possible. Place a second needle (or forceps) toward the posterior end to hold the larva in place (fig. 7.4).
4. Slowly slide the first needle away from the second. If this is done correctly, the mouthparts and salivary glands will be pulled loose from the body. The glands are transparent and elongate, whereas the digestive tract and trachea are much denser in appearance. A fat body usually runs along the sides of the glands and may help you identify them.
5. Move the glands to a clean slide or a clean area of the original slide. Dispose of the debris and remove the salt solution.
6. Put a drop of *aceto-orcein* stain on the glands and stain for 5–10 minutes. The glands should not be allowed to dry.
7. Cover with a coverslip and place in the fold of a paper towel. Use the end of your thumb to press firmly on the preparation to squash (do not break the glass) the glands into a thin smear. Do not slide the coverslip.
8. Scan under the low power objective to find the nuclei easily. Some cells should show a mass of chromosomes. Focus these cells with the high power objective to see the chromosomal banding.
9. A better view is possible if the nuclei are broken. Do this by tapping the coverslip gently with the handle of the dissecting needle. The chromosomes should be well spread but not broken. The slide should not be allowed to dry. The edges of the coverslip can be temporarily sealed with fingernail polish or wax.
10. Examine the prepared slide of *Drosophila* chromosomes and compare this to the slide you made.

Experimental Crosses in Plants (Optional)

Monohybrid Cross

In tobacco plants, there is a dominant allele (*C*) for chlorophyll that gives the plants a green color. If a plant is homozygous for the recessive allele (*c*), it cannot manufacture chlorophyll and appears white in color.

1. Obtain a numbered agar plate on which tobacco seedlings are growing. Record this number in table 7.6. The original parents (P generation) were homozygous. These seedlings are the offspring of the F_1 heterozygous plants. What are the expected phenotype results for the F_2 generation? _____
2. Using a binocular dissecting microscope to view the seedlings, count the number that are green and the number that are white.

3. Record your results for this plate in table 7.6. Do your results differ from the expected results? _____
4. Do a chi-square test to determine if the deviation from the expected results can be accounted for by chance alone. Chi-square test results:

Table 7.6 Tobacco Seedlings

Plate No.	Green	White

If the deviation is significant, count several other plates of tobacco seedlings and run the chi-square test on these data.

Dihybrid Cross

In this instance, the P generation plants were homozygous for 2 pairs of alleles, and the F_1 generation plants were heterozygous for these alleles.

1. Obtain an ear of corn from the supply table.
2. Record the sample number in table 7.7.

Table 7.7 Corn Kernels

Sample No.			
Yellow Smooth	**Yellow Rough**	**Purple Smooth**	**Purple Rough**

Notice that the kernels differ in color (either purple or yellow) and texture (either smooth or rough). ❸ Count how many kernels are yellow and smooth; yellow and rough; purple and smooth; purple and rough. ❸ Record your data in table 7.7. ❸ From these data, determine which of these traits are dominant and which are recessive. Dominant: _____ and _____ Recessive: _____ and _____ . If so directed, use the chi-square test to determine if your results are due to chance or some other factor.

Laboratory Review 7

_____ 1. List 2 sex distinguishing characteristics of fruit flies.

_____ Male: a.

_____ b.

_____ Female: c.

_____ d.

_____ 2. If the F_1 offspring exhibit a 3:1 ratio, what are the genotypes of the parents?

_____ 3. In fruit flies, which of the characteristics you studied was X-linked?

_____ 4. If the F_2 generation exhibits a 9:3:3:1 phenotypic ratio, what was the genotype of the parents?

_____ 5. If the F_2 results were 90 long wing flies to 30 vestigial wing flies, what were the phenotypes of the parents (P)?

6. Briefly describe the life cycle of *Drosophila*.

7. When you counted the tobacco seedlings in one agar plate, there were 40 green plants and 2 white plants. According to the chi-square test, do your results support the hypothesis that both parent plants were heterozygous for the color allele?

8. After counting the tobacco seedlings in 6 agar plates, your data are as follows: 125 green plants and 39 white plants. According to chi-square test, are your deviations from the expected values due to chance?

9. Students in the laboratory periods before you removed some of the purple and yellow kernels as they were counting. What effect will this have on your results?

References

Paul, Carol. 1972. *Principles of Biological Science*. Dubuque, IA: Kendall/Hunt Publishing Company.

Snow, B. L., ed. *Biological Laboratory Exercises*. Boston, MA: Suffolk University.

Laboratory

8

Human Genetics

Learning Objectives

Students should be able to

1. explain and prepare a human karyotype when provided with the necessary materials;
2. distinguish X-linked recessive alleles from autosomal alleles by the pattern of their inheritance;
3. write genotypes for X-linked recessive traits for each sex and do genetics problems that involve sex-linked genes;
4. predict from observing pedigree charts whether the characteristic in question is controlled by an autosomal dominant, recessive, or X-linked recessive allele;
5. suggest the most probable genotype by observing the phenotype when it is known whether the characteristic is dominant or recessive.

Human Chromosomes

Each species has a characteristic number of chromosomes; for example, corn cells have 20 chromosomes, mice cells have 40 chromosomes, and human cells have 46 chromosomes. To view the chromosomes clearly so that they can be counted, a cell may be treated and photographed just prior to dividing, as described in figure 8.1. The chromosomes may then be cut out of the photograph and arranged by homologous pairs. *Homologous chromosomes* are a pair of chromosomes that carry genes for the same trait. The resulting display of chromosomes is called a *karyotype*. A normal human male karyotype is shown in figure 8.2. Notice the 7 groups, A–G, in which the chromosomal pairs are placed.

Autosomal chromosomes are any of the 22 pairs (1–22) of chromosomes other than the sex determining chromosomes. The members of the pair contain genes governing the same traits, but they are not necessarily identical. The chromosomes of the twenty-third pair, the *sex chromosomes,* are dissimilar in the male: there is a larger X chromosome and a smaller Y chromosome. Females have 2 X chromosomes in their karyotypes.

Normal Human (Male) Karyotype

A normal human male karyotype is shown in figures 8.1 and 8.2. Note the dissimilar nature of the X and Y chromosomes. The X chromosome possesses more gene loci (positions) than the Y chromosome. Supporting evidence points to the Y chromosome as having the gene for maleness. No male individuals are born without a Y chromosome.

S 1. Using figure 8.2, measure the length of the X and Y chromosomes to the nearest millimeter, and enter the data following.

S 2. Divide the length of the Y by the length of the X.

S 3. Multiply this figure by 100 to compute the percentage the Y gene is of the X gene.

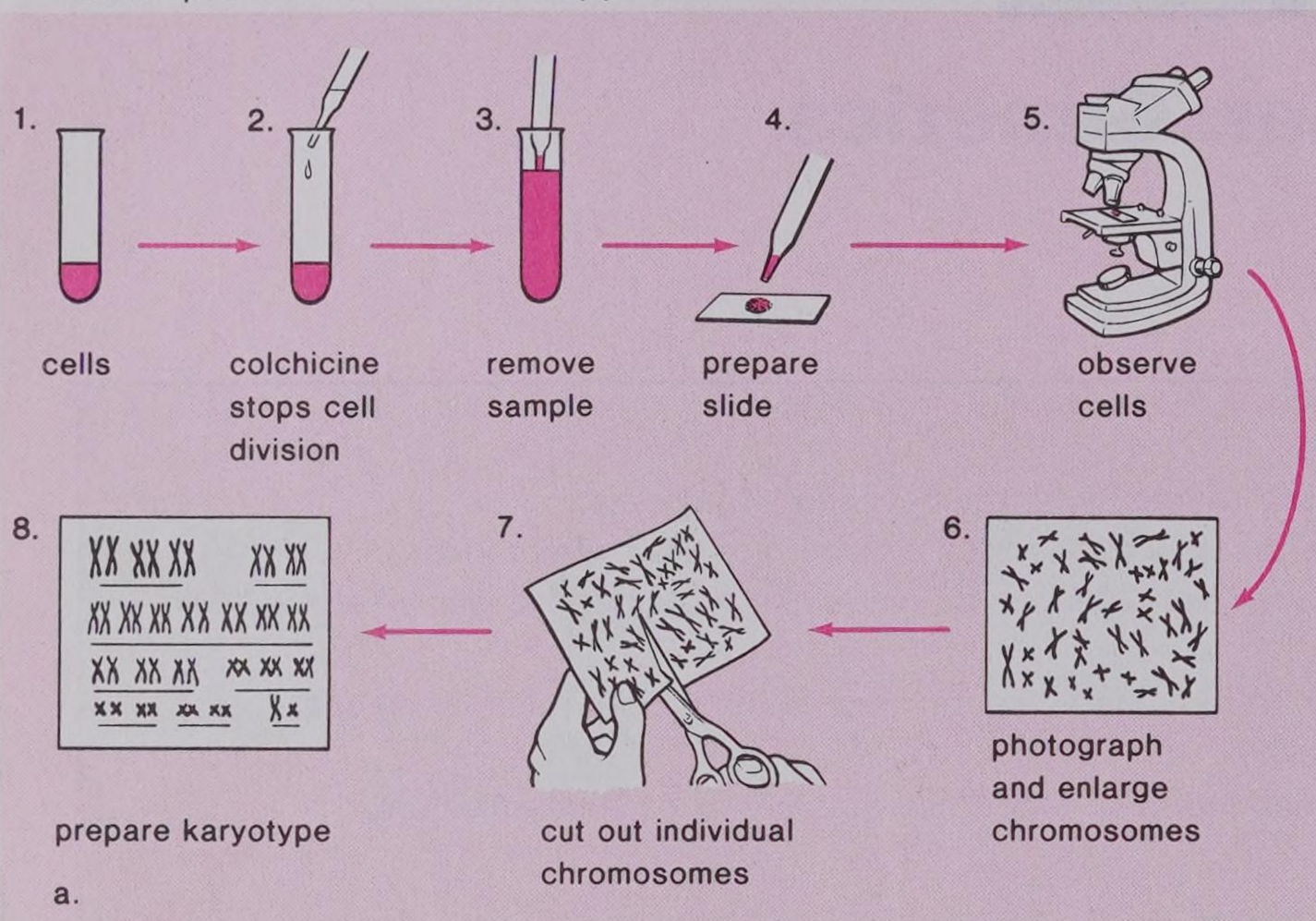
Percentage length of the Y chromosome to the X chromosome:

$$\frac{\text{Length of Y chromosome (mm)}}{\text{Length of X chromosome (mm)}} \times 100 = \text{——} \times 100 =$$

Abnormal Karyotypes

Occasionally, individuals are born with abnormal karyotypes. Several abnormalities are listed in table 8.1. These abnormalities may occur from the following:

Figure 8.1 Human karyotype. ***a.*** Simplified version of karyotype preparation. Between steps (2) and (3) are 2 additional important steps: swelling the cells in hypotonic salt solution to facilitate spreading of the chromosomes and fixation of the cells (by alcohol and acetic acid) to kill them. Between steps (4) and (5) the cells must be stained for chromosomes to be visible. ***b.*** These chromosomes of a human male were prepared for microscopy by G-staining, which provides the banded appearance of chromosomes. The bands are used in karyotype analysis.



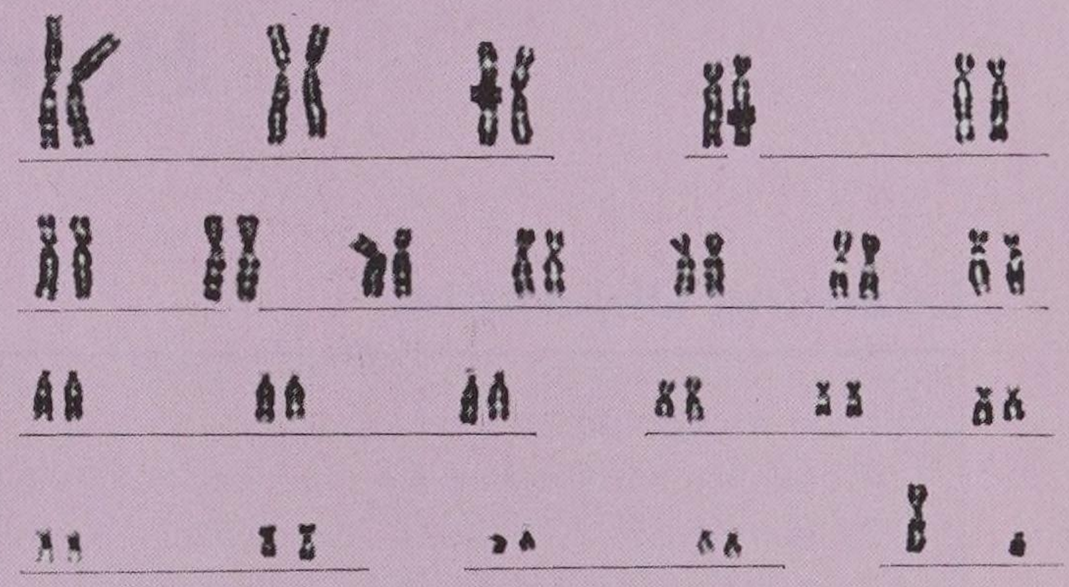
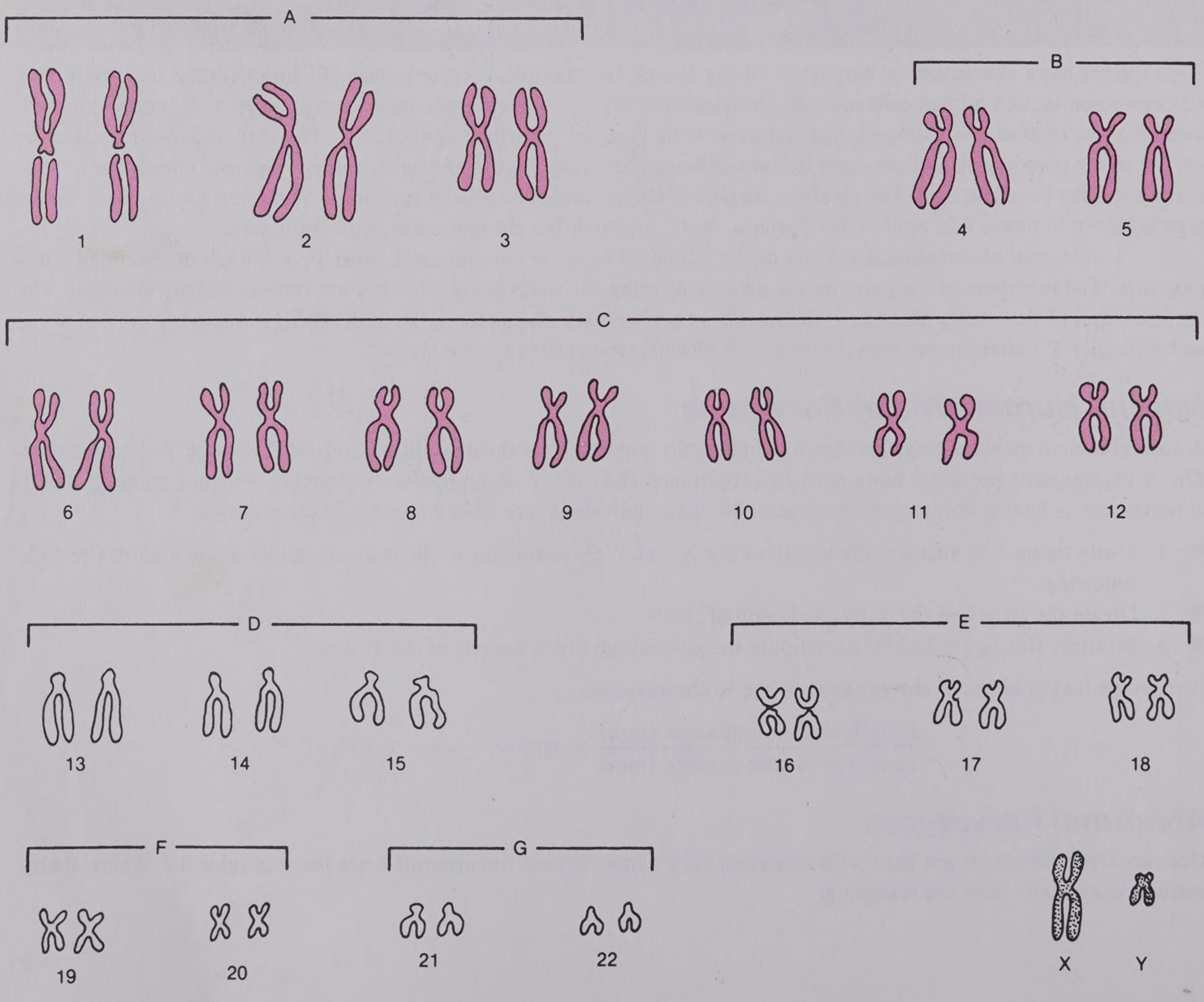


Figure 8.2 Normal human (male) karyotype.



Deletion: a segment of a chromosome is missing.
Duplication: a segment of a chromosome is repeated.
Inversion: a segment of a chromosome is turned around 180°.
Translocations: segments of 2 chromosomes are interchanged.
Aneuploidy: there is an excess or a deficiency of single chromosomes in an otherwise normal diploid cell with 46 chromosomes. An excess (i.e., 3 chromosomes in a homologous set) is called trisomy. A deficiency (i.e., only one chromosome in a homologous set) is called monosomy.

Table 8.1 Interpretation of Karyotypes

Chromosomal Makeup	Clinical Conditions
46 XX	Female (normal).
46 XY	Male (normal).
13 trisomy (3 number 13 chromosomes)—Patau syndrome	D_1 syndrome. Multiple congenital anomalies; fatal by 1 year in most cases.
18 trisomy—Edward syndrome	E syndrome. Multiple congenital anomalies; fatal by age 3 months in most cases.
G trisomy	Down syndrome. Mental retardation and distinctive facial characteristics (e.g., skin fold at inner corners of eyes).
XXY	Klinefelter syndrome. Male appearance; testes underdeveloped, breasts enlarged. Usually sterile, often mentally retarded.
XO	Turner syndrome. Female, anatomically and physiologically; rudimentary ovaries, no menstruation or ovulation. (YO male condition does not seem to be a viable one. None have been found.)
XXX, XXXX, XXXXX, XXXY, XXXXY	Multiple sex chromosomes. Some degree of mental retardation, some degree of anatomical or physiological abnormality, or both. Individuals may be fertile; may produce normal offspring.
D-G translocation	Small fragments are broken off one 15 (D) and one 21 (G) chromosome, and the damaged chromosomes fuse together. Individuals in which this occurs will be normal themselves but will produce some gametes containing both the D-G translocation and chromosome 21 (G). If such a gamete is involved in zygote formation, the embryo will be affected with G trisomy (Down syndrome).
G-G translocation	Similar to D-G translocation. (Chromosome 22 instead of 15 is involved.)
Partial deletion short arm B	Cri-du-chat syndrome. Rounded, moonlike faces; feeble, plaintive catlike cries; mental and physical retardation.
Partial deletion short arm 18	Found in cases of mental retardation and congenital malformations.
Partial deletion long arm G	Philadelphia chromosome. Found in patients with chronic myeloid leukemia. Involves one 22 (G) chromosome.
Partial deletion long arm Y	Found in some cases of infertility.

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Down Syndrome

Down syndrome, with 3 number 21 chromosomes (called G trisomy in table 8.1), is the most common abnormality observed.

1. Using the representations of the chromosomes provided (fig. 8.3) construct a karyotype for a person with Down syndrome. (Notice that the sex chromosomes are stippled.)
2. Using the *sticky pages* of chromosomes at the end of this manual, arrange them on the analysis sheet provided.
3. Match the pairs and put them in groups, referring to figure 8.2 for guidance.

Unknown Abnormality

1. Using the same procedure as before, construct another karyotype (fig. 8.4).
2. Use table 8.1 to diagnose the abnormality of this particular human being.

Which condition does this individual appear to have? _____

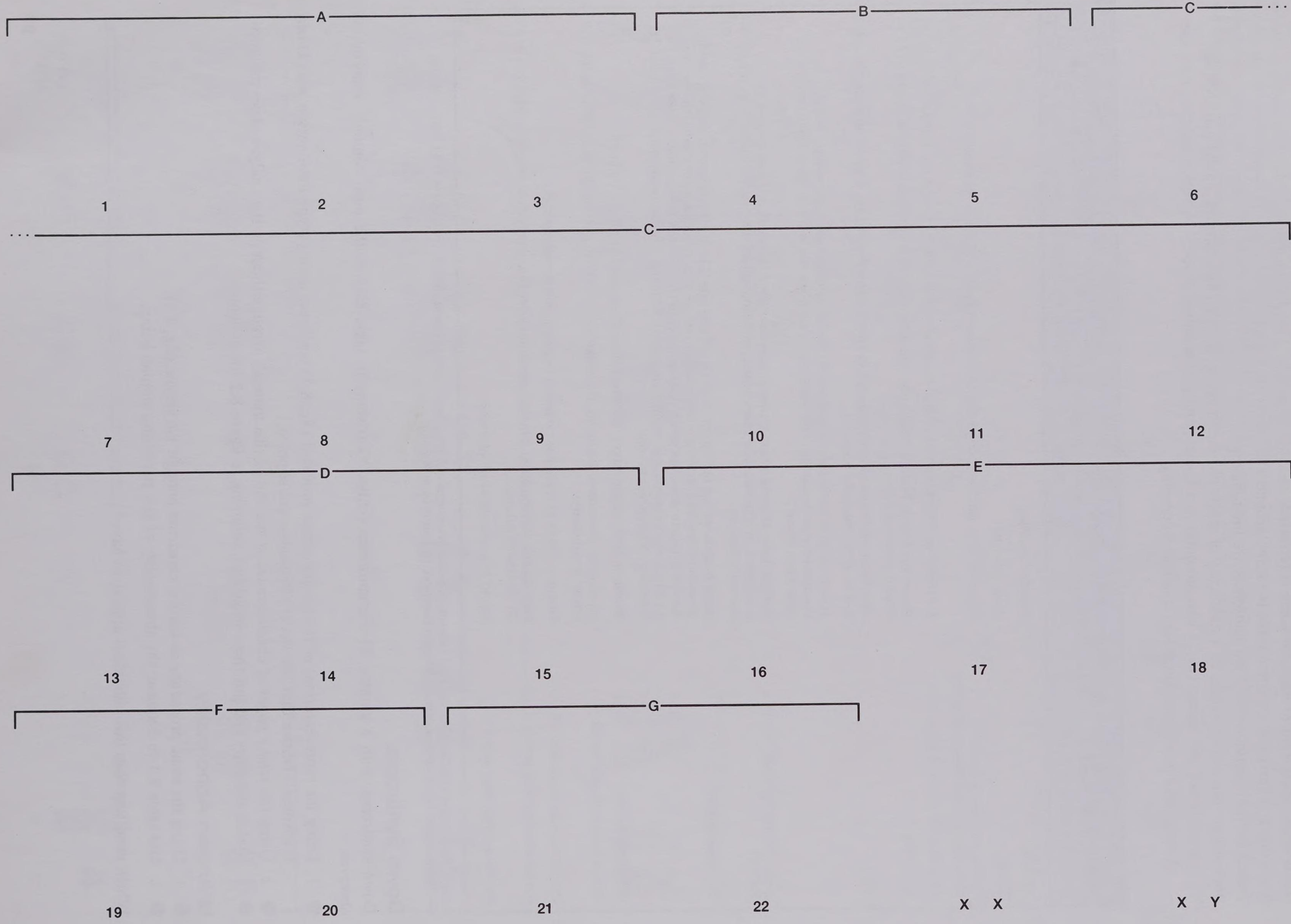


Figure 8.3 Karyote analysis sheet—Down syndrome.

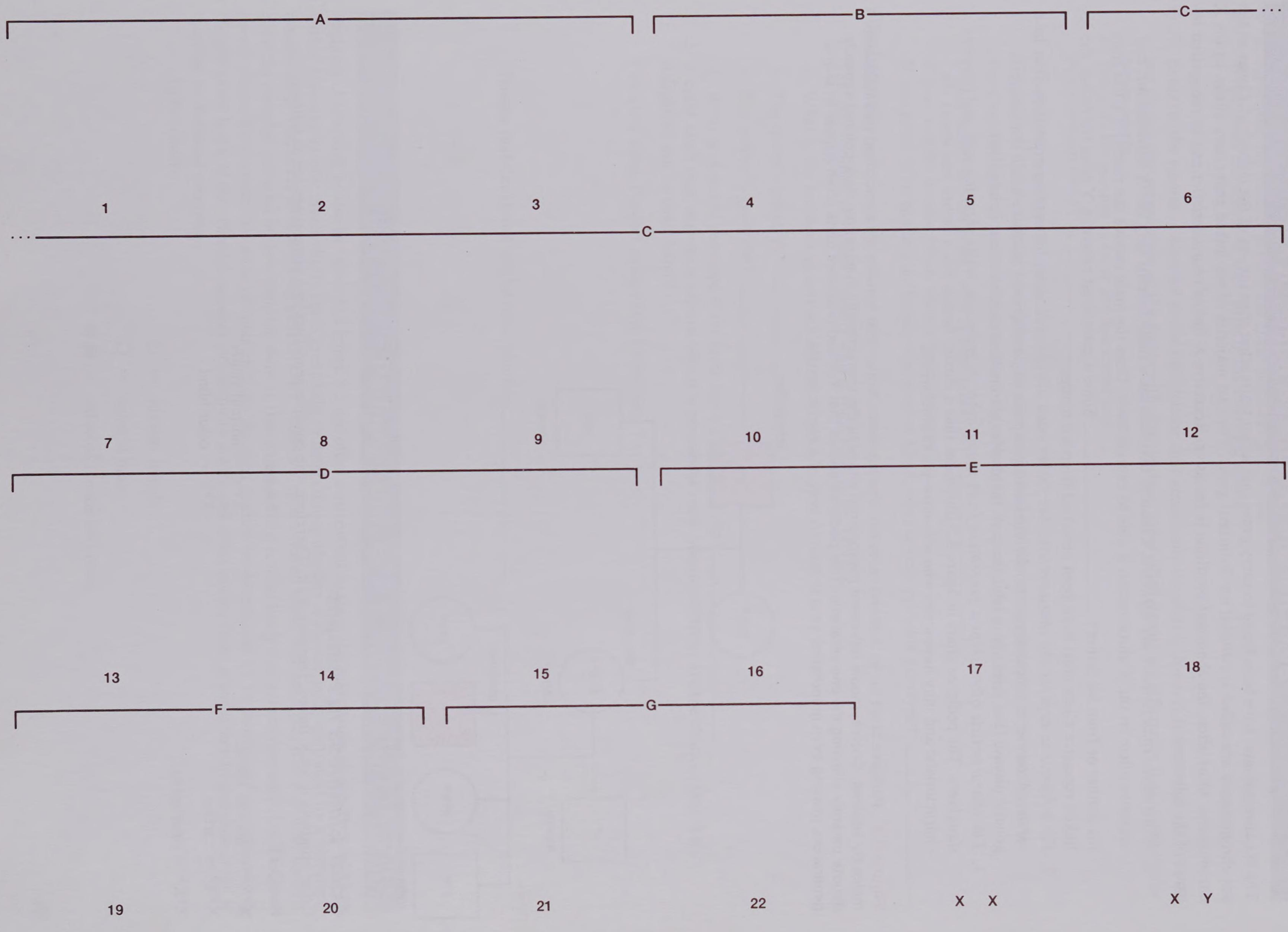


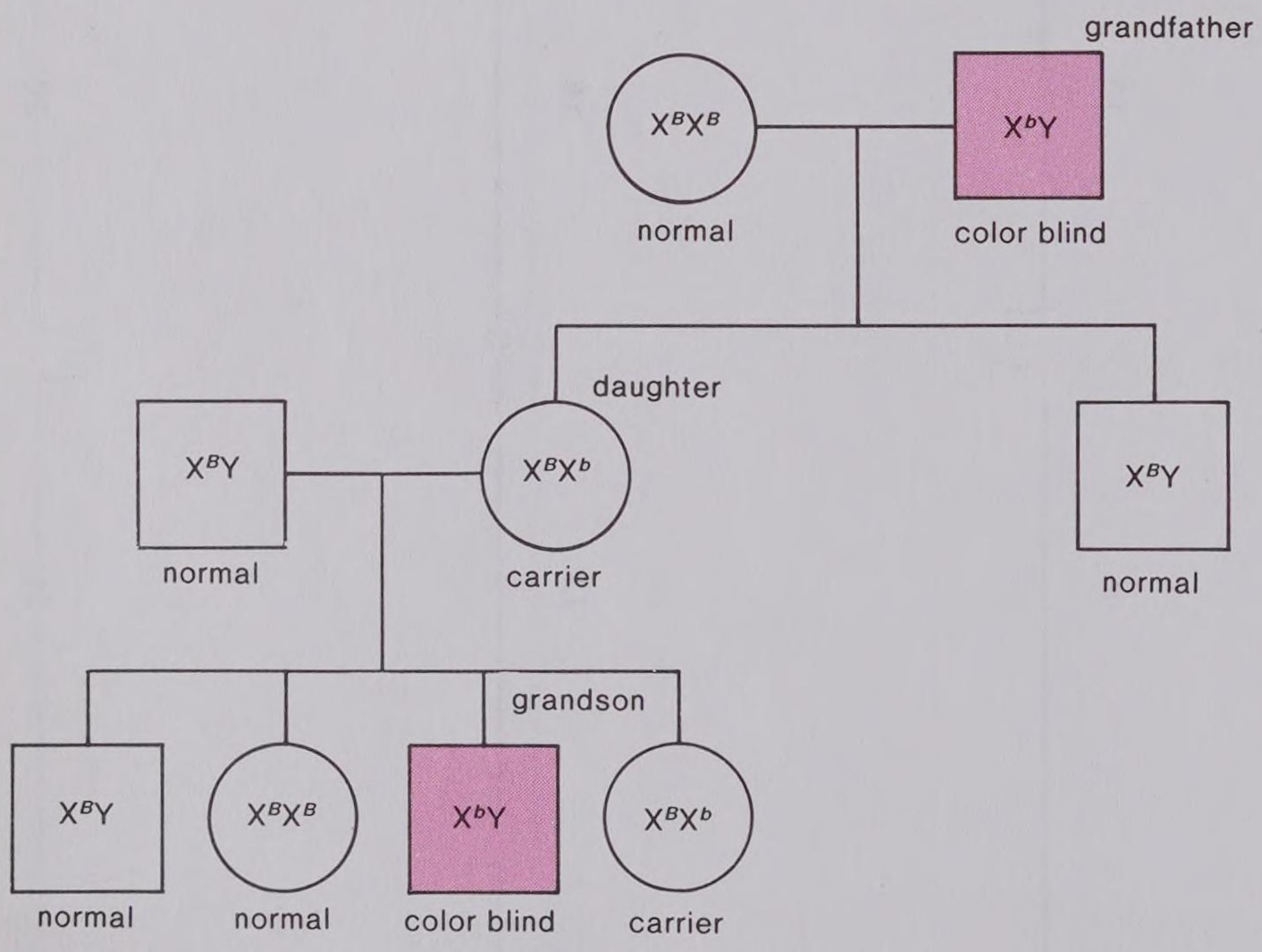
Figure 8.4 Karotype analysis sheet—unknown.

Sex Linkage (X-Linked Recessives)

The sex chromosomes have been found to carry genes that affect traits other than the sex of the individual. Genes on the sex chromosomes are called *sex-linked* (or X-linked) *genes*. The vast majority of sex-linked genes have alleles on the X chromosome. Most often, the abnormal condition is recessive. Recessive *X-linked characteristics* can be recognized because of the following:

1. *More males than females are afflicted.* Consider that because a male inherits only one X chromosome, any recessive allele on the X chromosome is able to express itself. Does the male receive the recessive allele from his mother or from his father? _____ Since a male must receive a Y from his father, the X-linked recessive allele must have been inherited from his mother.
2. *For a female to express the characteristic, her father must also have it.* Also, her mother must show it or be a carrier. A *carrier* is a heterozygous individual who is capable of *passing* on a recessive allele that causes a genetic disease (but does not exhibit the trait themselves), a medical condition that is inherited.
3. *The characteristic often skips a generation from grandfather to grandson, while skipping sons and (carrier) daughters.* The pedigree chart in figure 8.5 illustrates that a carrier female has a father who shows the characteristic and may have a son who will show the characteristic.

Figure 8.5 Pedigree chart for an X-linked recessive characteristic. Notice that females are symbolized by circles and males by squares. Open symbols represent persons not showing the characteristic in question; dark-colored symbols denote persons showing the characteristic. This pedigree shows that X-linked recessive traits often appear to skip a generation, passing from grandfather to grandson by way of a carrier female.



Color Blindness in Humans

Color blindness is an X-linked, recessive trait. Therefore, the possible genotypes and phenotypes are as follows:

Females

X^BX^B = normal vision

X^BX^b = carrier

X^bX^b = color blind

Males

X^BY = normal vision

X^bY = color blind

Your Genotype

1. Have your lab partner present the color blindness test to you.
2. Write down the results of each page in the following space.

3. Evaluate the results as directed in the instructions that accompany the charts. *Note:* It is important that you *do not* discuss the appearances of the plates with your partner; color-blind people see differently than normal color vision people.
4. Test your lab partner as he or she has tested you.

Are you color blind? _____

What is your genotype? _____

If you are a female and not color blind, you may judge whether you are homozygous or heterozygous by knowing whether any member of your family is color blind (hypothetical question).

If your father is color blind, what is your genotype? _____

If your mother is color blind, what is your genotype? _____

If you know of no one in your family who is color blind, what is your probable genotype? _____

Problems

1. The only member of Arlene's family who is color blind is her brother.
   a. What is the brother's genotype? _____
   b. The father's genotype? _____
   c. The mother's genotype? _____
   d. What is Arlene's genotype if she later has a color-blind son? _____
2. A color-blind man marries a woman who is a carrier for color blindness. What are the chances that their daughters will be color blind?

Calculate using Punnett square (see Laboratory 7 or text):

Chances that daughters will be color blind: _____

Pedigree Charts

Pedigree charts such as the one shown in figure 8.5 are often constructed to show the inheritance of genetic conditions within a human family. Such charts are a great help in determining whether a phenotype is controlled by a dominant (and it never reappears in succeeding generations), recessive, or sex-linked allele. For example, if one or both parents show the disorder, yet none of their children show it, that characteristic is most likely *autosomal dominant*. Or, if neither parent shows the disorder but some of their children do, then it must be determined by an *autosomal recessive* allele. On the other hand, if the disorder appears primarily in males and often passes from grandfather to grandson, then it must be an *X-linked recessive*.

In the charts:

○ = normal female

□ = normal male

●■ = affected female and male

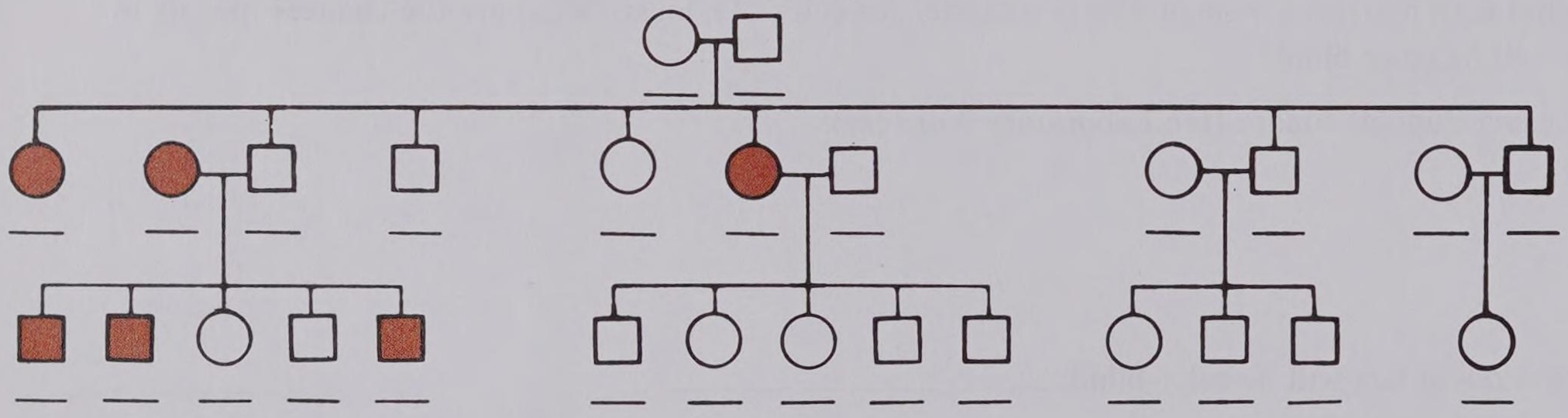
A carrier is an individual who appears to be normal but who is capable of passing on a gene for the disorder. If the characteristic is dominant, there can be no carriers because only a single gene is needed to show the disorder.

Practice Pedigree Charts

1. For each pedigree, using the information given previously and in table 8.2, state your opinion as to whether the disorder in question is controlled by an autosomal dominant or recessive allele, or whether it is controlled by an X-linked recessive allele.
2. Choose a key from table 8.2 for each trait, and write the *genotype* (as far as it can be determined) below each individual.

Table 8.2 Pedigree Chart

Characteristic	Possible Keys	Key No.
Autosomal dominant	*AA* = affected *Aa* = affected *aa* = normal	1
Autosomal recessive	*AA* = normal *Aa* = carrier *aa* = affected	2
X-linked recessive	X^AX^A = normal female X^AY = normal male X^AX^a = carrier female X^aX^a = affected female X^aY = affected male	3

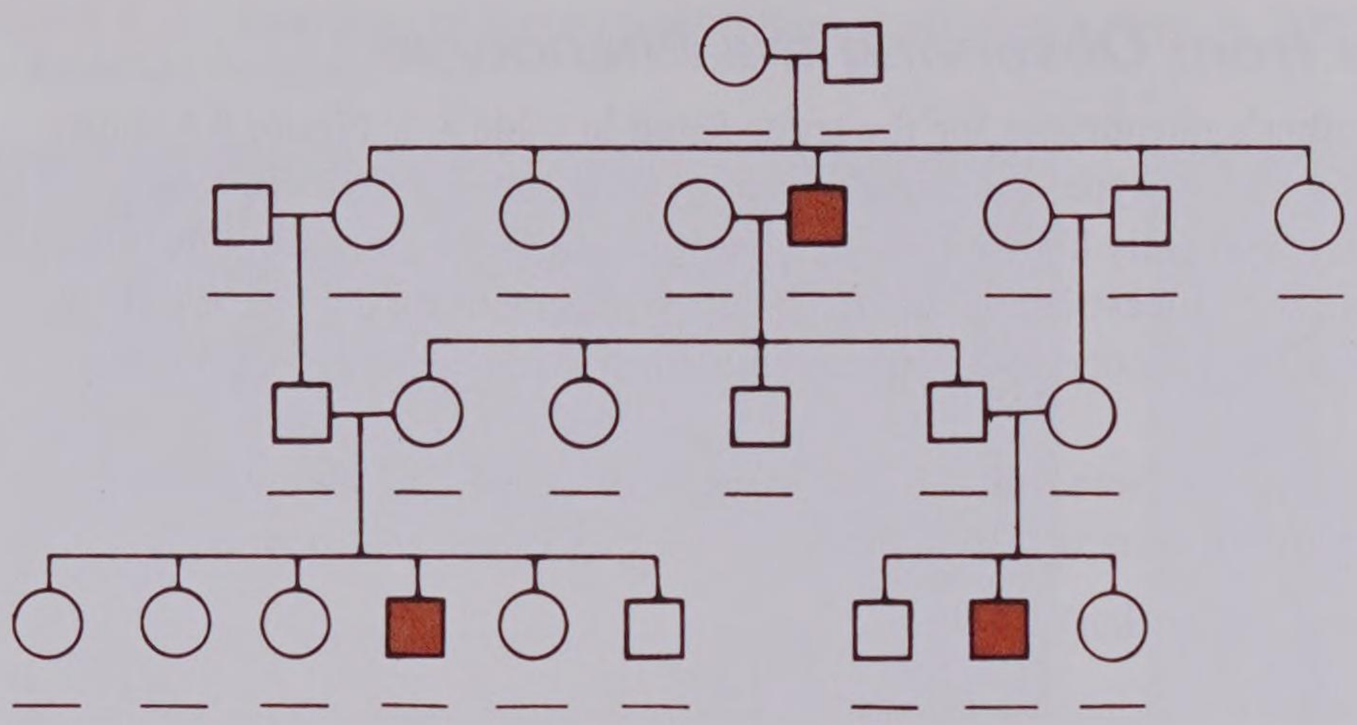


Pedigree number 1

1. a. How is this disorder inherited? _____

   b. What key number from table 8.2 do you suggest? _____

   c. Write in the genotype for each individual.

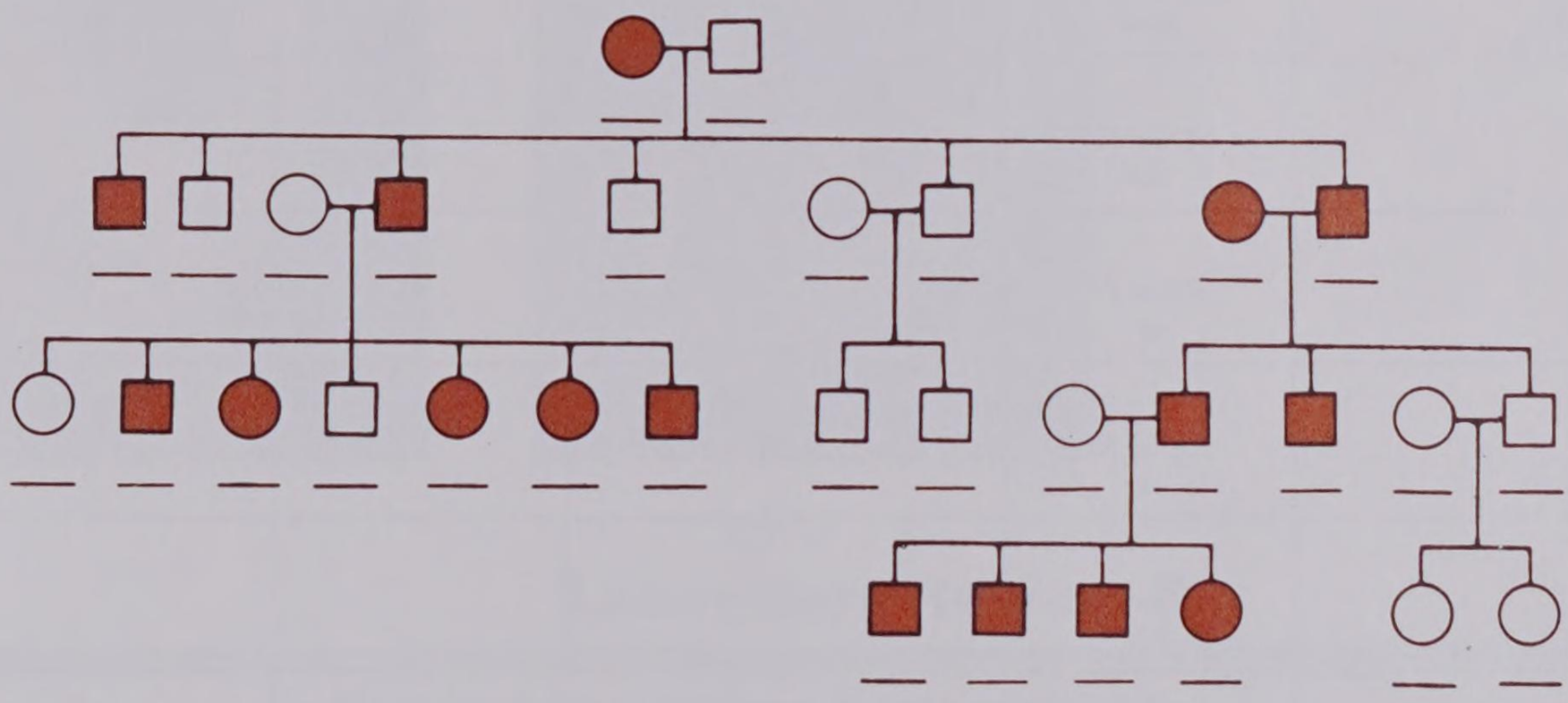


Pedigree number 2

2. a. How is this disorder inherited? _____

   b. What key number from table 8.2 do you suggest? _____

   c. Write in the genotype for each individual.



Pedigree number 3

3. a. How is this disorder inherited? _____

   b. What key number from table 8.2 do you suggest? _____

   c. Write in the genotype for each individual.

Determining Human Genotypes from Observing the Phenotype

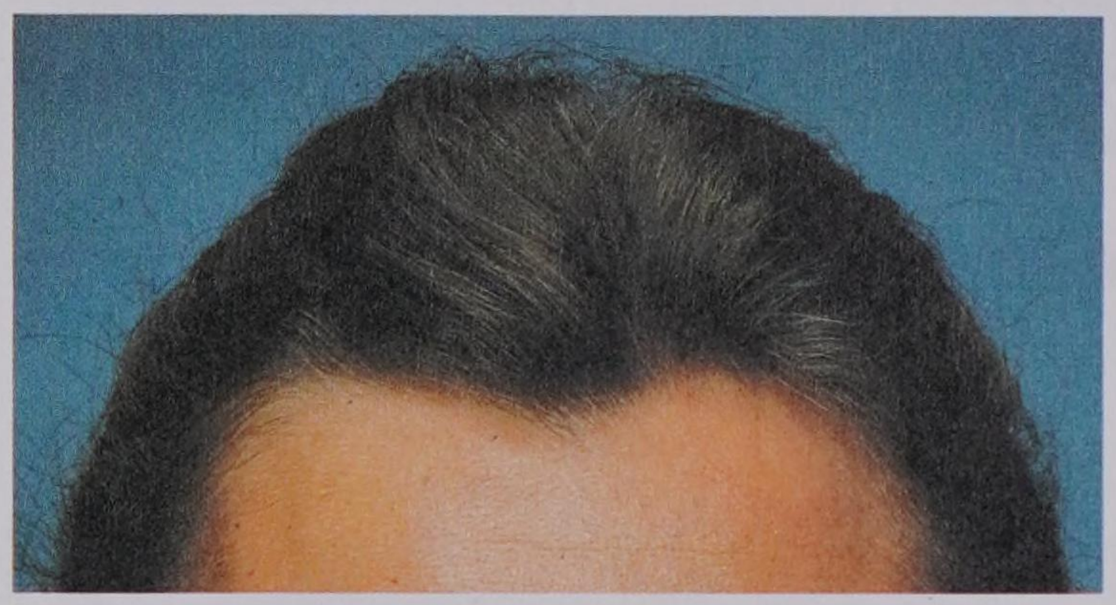
1. Choose a lab partner and determine each other's phenotypes for the traits listed in table 8.3. Figure 8.6 shows characteristics for 2 of the traits. *Record* your phenotype and probable genotype in the chart. If you have a recessive characteristic, your genotype is automatically known (homozygous recessive). If you have a dominant characteristic, it is always possible to determine at least one allele. If you know that you are dominant but cannot determine your genotype by recalling the phenotype of your parents, brothers and sisters, or children, list it as A^{-}.
2. Your instructor will tally the class's phenotypes for these traits so that you can complete the column entitled Number in Class. Calculate the percentage of the class with each characteristic. Is it true that dominant phenotypes are always the most common in a population? Explain. _____

_____

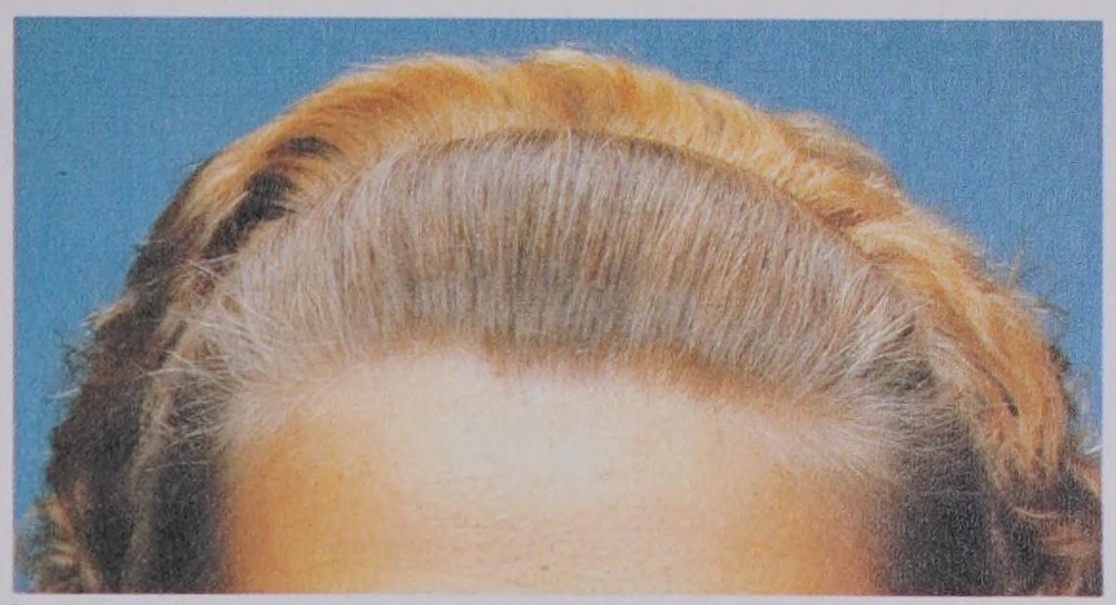
Table 8.3 Human Traits

Characteristic: d = Dominant, r = Recessive	Possible Genotypes	Personal Phenotype and Genotype	Number in Class	Percentage of Class with Trait
Hairline widow's peak (d) continuous hairline (r)	*WW* or *Ww* *ww*			
Earlobes unattached (d) attached (r)	*UU* or *Uu* *uu*			
Skin pigmentation freckles (d) no freckles (r)	*FF* or *Ff* *ff*			
Color vision red-green vision (d) red-green blindness (r)	*RR* or *Rr* *rr*			
Hair on back of hand present (d) absent (r)	*HH* or *Hh* *hh*			
Thumb hyperextension last segment cannot be bent backward (d) last segment can be bent back to 60° (r)	*TT* or *Tt* *tt*			
Bent little finger little finger bends toward ring finger (d) straight little finger (r)	*LL* or *Ll* *ll*			
Interlace fingers left thumb over right (d) right thumb over left (r)	*ll* or *li* *ii*			

Figure 8.6 Examples of human phenotypes. ***a.*** Widow's peak. ***b.*** Continuous hairline. ***c.*** Unattached earlobes. ***d.*** Attached earlobes.

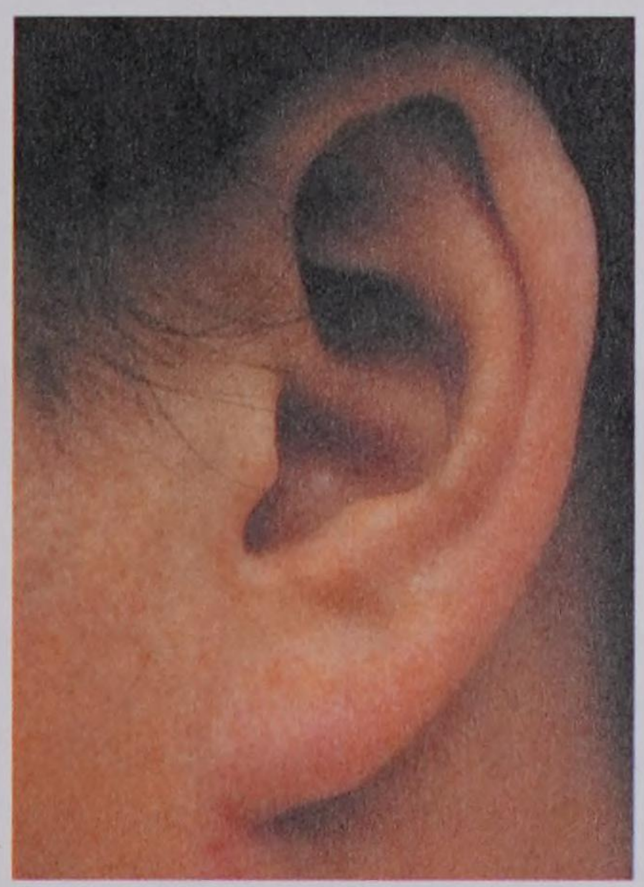


a.



b.

c.



d.

Laboratory Review 8

_____ 1. What are the sex chromosomes for a normal male?

_____ 2. a. If a man is color blind, what is his genotype?

_____ b. What is his father's genotype?

_____ 3. If neither parent shows the characteristic, but children of both sexes do, what is the pattern of inheritance?

_____ 4. A woman who is color bind has 6 sons. How many of them will be color blind?

_____ 5. What is the syndrome if a person has a karyotype with the chromosomes XXY?

_____ 6. In humans, the ability to taste PTC (*A*) is dominant to the gene for inability (*a*). The brown-eyed gene (*B*) is dominant to blue (*b*).

_____ a. What proportion of the offspring of 2 parents, each of the genotype *AaBb*, would be blue-eyed tasters?

_____ b. brown-eyed tasters?

Reference

Volpe, E. P., and DeGruy, C. V. 1983.*Biology and Human Concerns Laboratory Manual,* 3d ed. Dubuque, IA: Wm. C. Brown Publishers.

Laboratory

9

Molecular Genetics

Learning Objectives

Students should be able to

1. describe the structure of DNA and RNA;
2. explain the method by which DNA replicates;
3. describe protein synthesis in terms of transcription and translation;
4. explain how each experimental procedure performed supports the central dogma of biochemical genetics.

Introduction

The molecular basis of inheritance has been found to depend on *DNA* (deoxyribonucleic acid). DNA is a polymer (a chain of molecular subunits) of nucleotides (fig. 9.1). Each one of these is composed of 3 parts: a 5-carbon sugar (deoxyribose), a phosphate group, and a nitrogenous base. There are 4 different types of nucleotides in DNA because there are 4 possible bases: adenine (A), guanine (G), cytosine (C), and thymine (T). DNA is double-stranded and exists as a double helix resembling a twisted ladder. The sides of the ladder contain only sugar-phosphate molecules, while the rungs are 2 bases hydrogen-bonded to each other. A is always paired with T, and C is always paired with G. This so-called complementary base pairing aids replication, when an exact copy of DNA is made. The double helix unwinds, and each nucleotide pairs with a new complementary nucleotide, forming 2 new complementary strands from the open templates of the old strands.

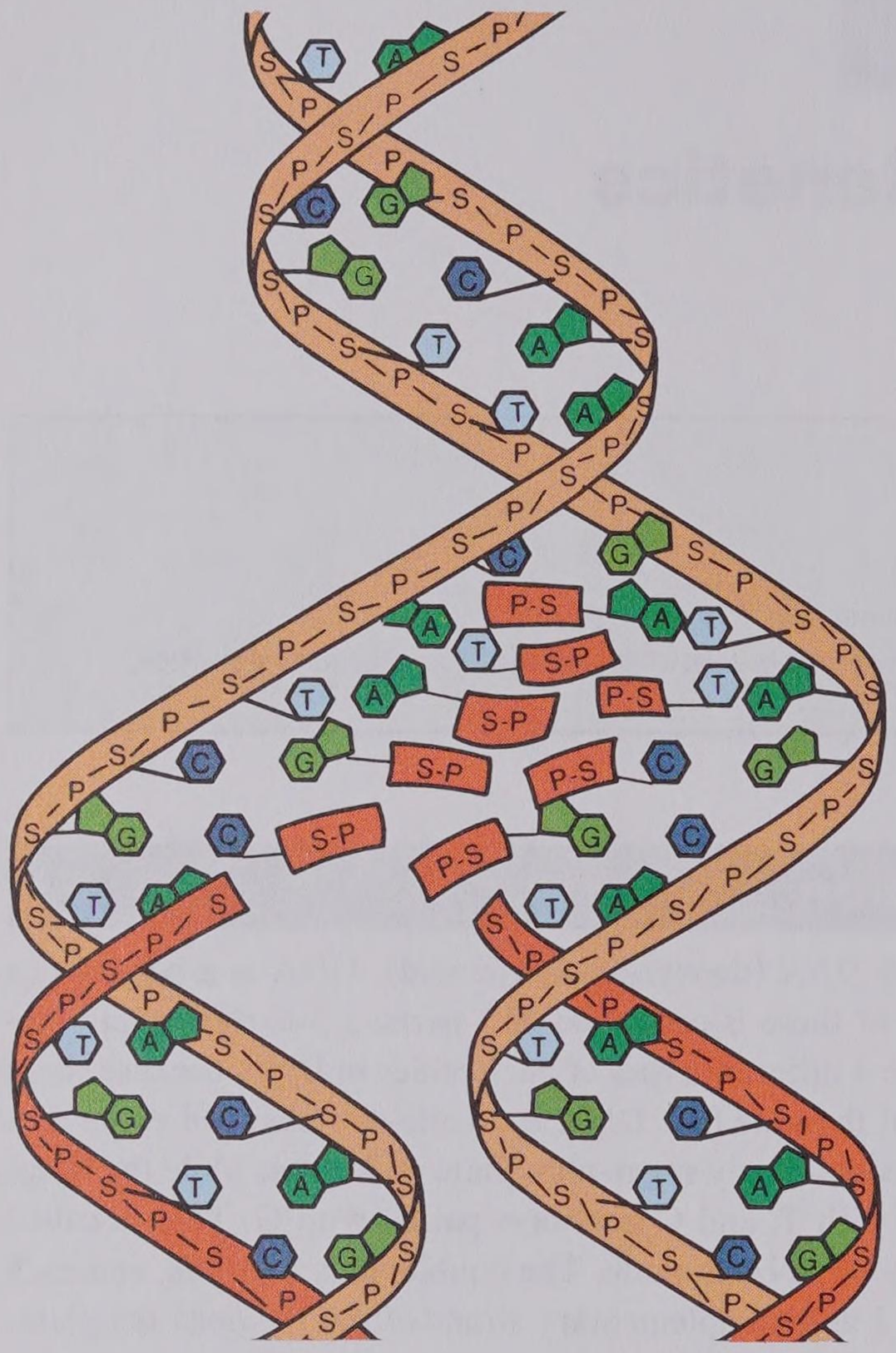
DNA not only serves as a template for its own replication but is also a template for *RNA* (ribonucleic acid) production. RNA differs from DNA in having a different sugar (ribose instead of deoxyribose), in substituting *uracil* (U) for *thymine* (T), and in being single-stranded. Actually, RNA exists in several distinct forms: messenger RNA (mRNA), ribosomal RNA (rRNA), and transfer RNA (tRNA). All of these have different functions in protein synthesis.

The nucleotide sequence in DNA serves as a template to form the complementary, single-stranded mRNA. In mRNA, each sequence of 3 nitrogenous bases serves as directions to add one specific amino acid to the protein that is being assembled. This triplet of 3 nitrogenous bases is a *codon.* The process of the formation of mRNA from the DNA template is *transcription.*

The mRNA strand passes from the cell nucleus (in eukaryotic cells, prokaryotic cells have no nuclear envelope defining a nucleus) out into the cytoplasm, carrying the instructions for the sequencing of amino acids in one polypeptide. Located in the cytoplasm are small molecules of tRNA.

The tRNA bears a specific nitrogenous base triplet *anticodon* (fig. 9.2), which is complementary to a mRNA codon. Each tRNA has a specific anticodon and an attachment site for one of the 20 amino acids. As ribosomes (containing rRNA) pass along the mRNA, amino-acid-bearing tRNA molecules pair complementarily with the codons (see fig. 9.3). When the amino acids have joined together, a polypeptide has formed. This process of converting the mRNA into an amino acid sequence is *translation.* The process of replication, translation, and transcription, is known as the *central dogma* of molecular genetics (see fig. 9.4).

Figure 9.1 Replication of DNA as proposed by Watson and Crick. In this simplified view, the 2 strands of a DNA molecule separate and each serves as template for the formation of a new complementary strand. While this is basically correct, the actual process has complications.



Region of parental DNA helix. (Both backbones are light.)

Region of replication (simplified). Parental DNA is unwound and unzipped. New nucleotides are pairing with those in parental strands.

Region of completed replication. Each double helix is composed of an old parental strand (*light*) and a new daughter strand (*dark*). Notice that each double helix is exactly like the other and also like the original parental double helix.

Figure 9.2 Structure of a transfer RNA (tRNA) molecule. Complementary base pairing indicated by dashes occurs between nucleotides that make up the molecule, and this causes the molecule to form its characteristic loops. The anticodon that pairs with a particular mRNA codon in a complementary manner occurs at one end of the folded molecule and the appropriate amino acid is attached at the other end of the molecule.

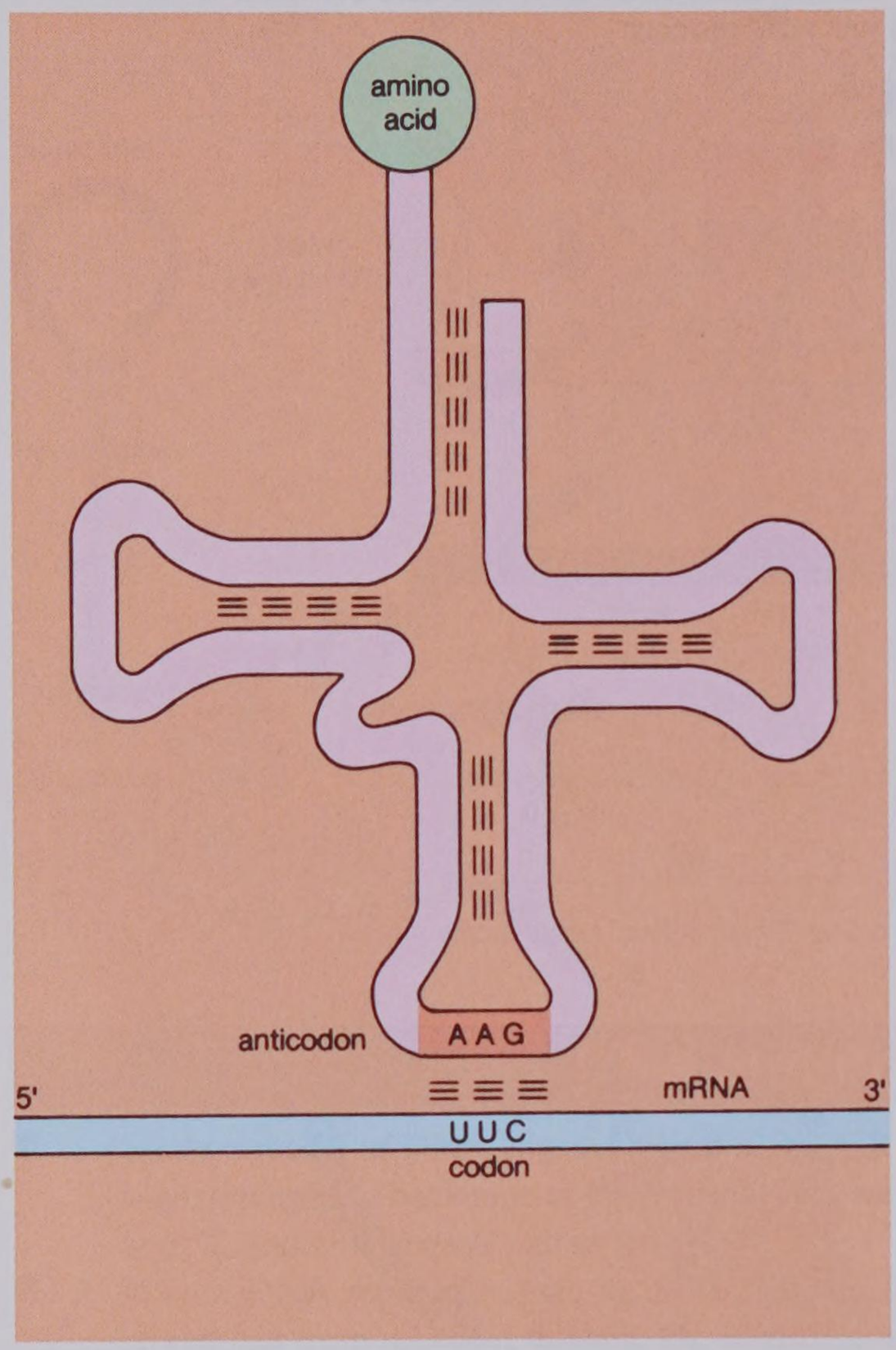


Figure 9.3 Chain elongation during protein synthesis. ***a.*** A tRNA bearing a peptide chain is already positioned on a ribosome (at the P site). ***b.*** A tRNA with the anticodon UUU recognizes its codon and binds to it (at the A site). ***c.*** The peptide chain is transferred and a peptide bond is formed with the new amino acid. As translocation occurs, a tRNA leaves. ***d.*** The tRNA bearing the peptide chain is now at the P site, and another tRNA amino acid complex is approaching the ribosome. The same sequence of events (***a–d***) will now reoccur.

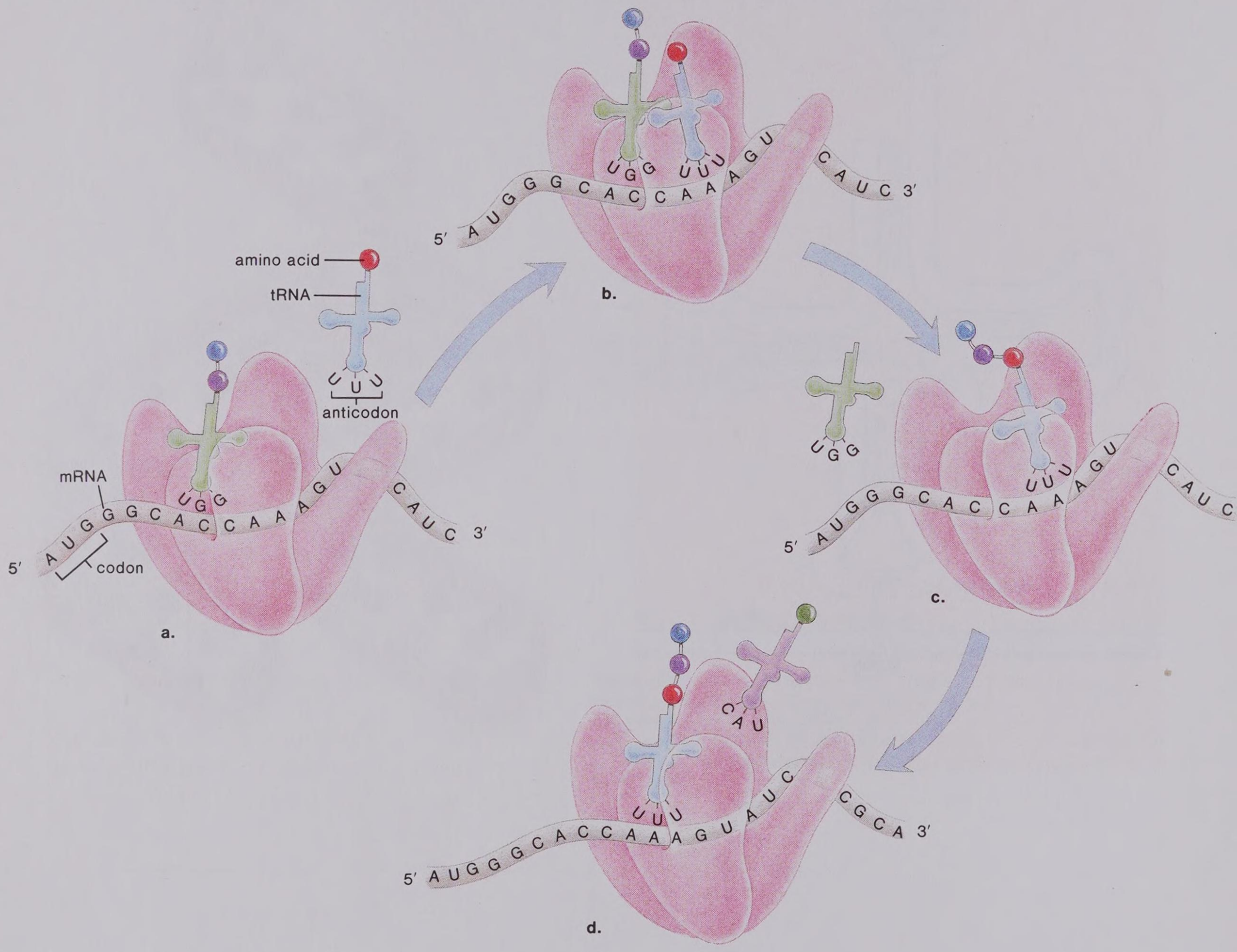
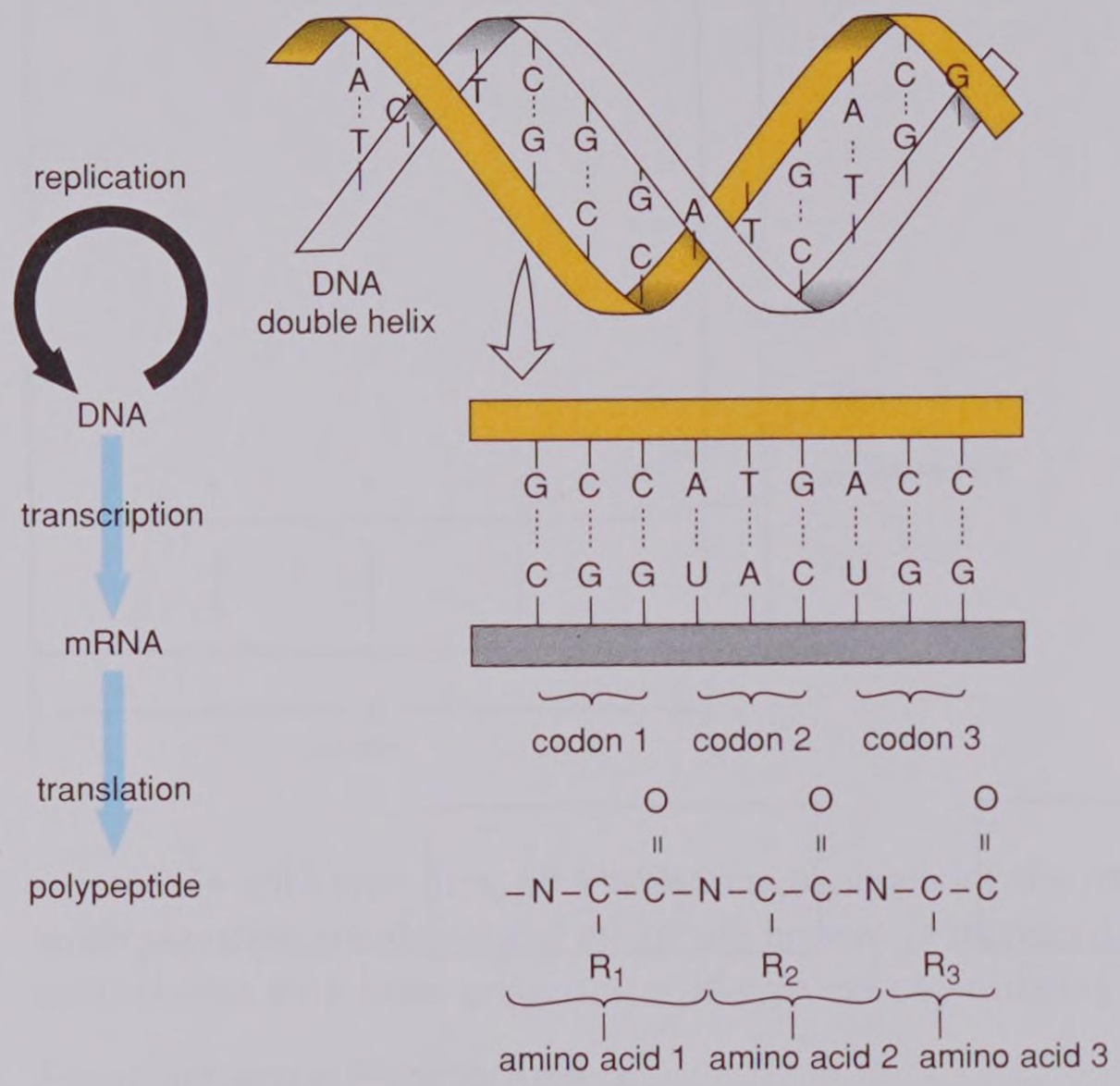

amino acid
tRNA
U U U
anticodon
mRNA
5′ A U G G G C A C C A A A G U C A U C 3′
codon
U G G
a.
5′ A U G G G C A C C A A A G U C A U C 3′
b.
U G G
c.
5′ A U G G G C A C C A A A G U A U C C G C A 3′
U U U
C A U
d.

Figure 9.4 Transcription and translation in protein synthesis. Transcription occurs when DNA acts as a template for RNA synthesis. Translation occurs when the sequence of codons (groups of 3 bases) carried by a type of RNA called messenger RNA (mRNA) dictates the sequence of amino acids in a polypeptide.



DNA and Protein Synthesis

1. Study a model of DNA (fig. 9.1); or if a DNA-RNA model is available, construct a DNA molecule. Notice the sugar-phosphate backbone of each strand. Notice the complementary base pairing and that A is always paired with T, and G is always paired with C.
2. Review DNA replication (see fig. 9.1). Take the 2 strands of the model apart and notice that a complementary strand can form adjacent to each. Or, if using a kit, actually construct new complementary strands for each of the original strands. Why is this form of DNA replication termed semiconservative (one-half new, one-half old)?

_____

_____

3. Review the process of transcription (preceding text and fig. 9.4). Adjacent to the DNA strand in figure 9.5, show a complementary mRNA strand. If you are using a kit, actually construct the mRNA molecule.
4. Review the process of translation (preceding text and figs. 9.3 and 9.4). (Remember, Uracil (U) substitutes for thymine (T) in all RNAs. Refer to your text for a table of all of the mRNA codons. This way you can confirm that your transcription and translation are correct.)
5. Fill in both the codons and anticodons in figure 9.6. If you are using a kit, actually construct the necessary tRNA molecules and the resulting tripeptide. (Correctly orient your mRNA strand by noting the position of the asterisk at one of its ends.)

Figure 9.5 Transcription diagram.

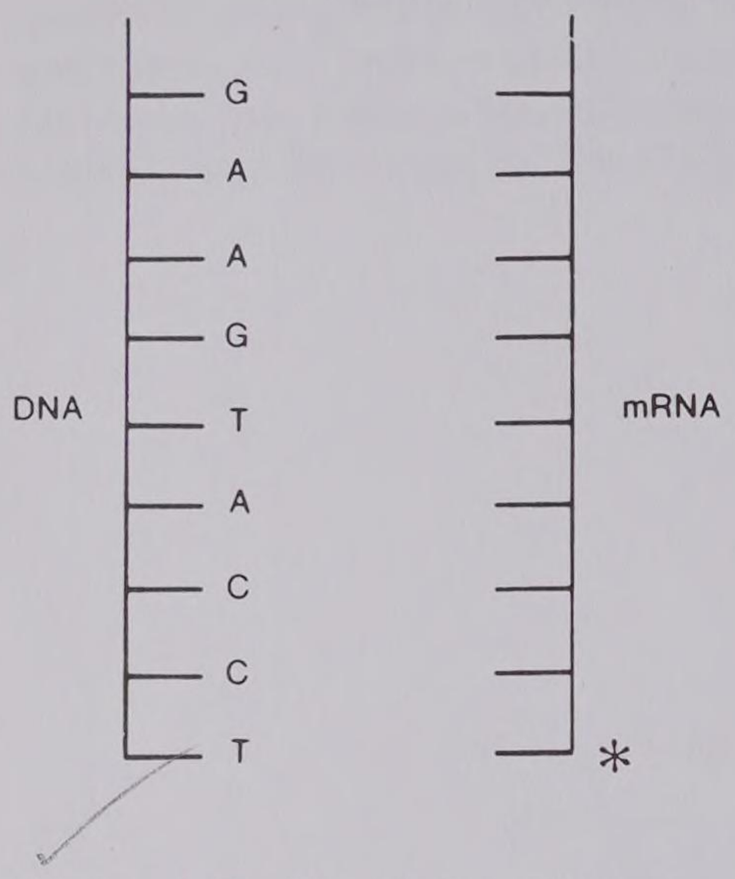
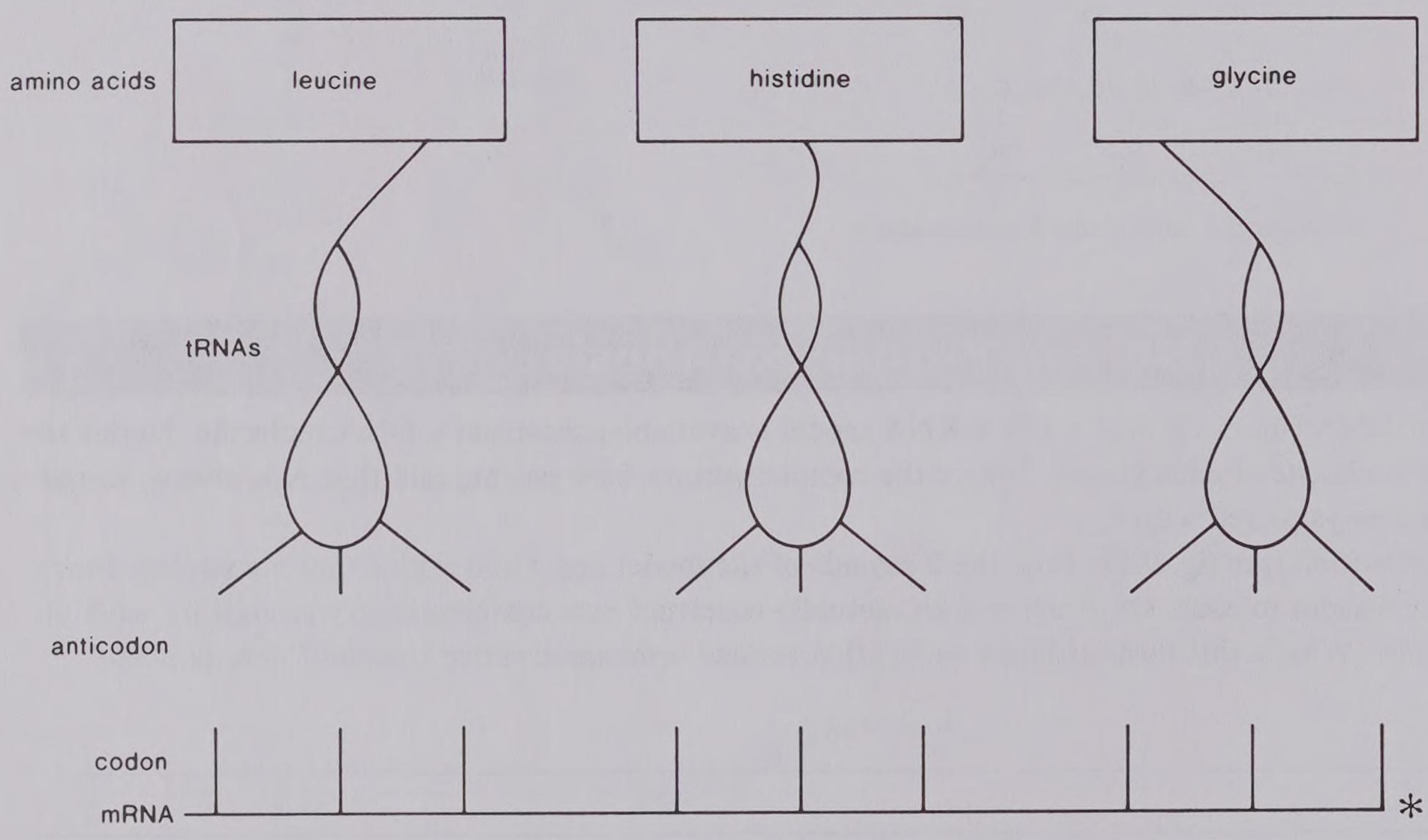


Figure 9.6 Translation diagram.



Relationship of Genotype and Phenotype

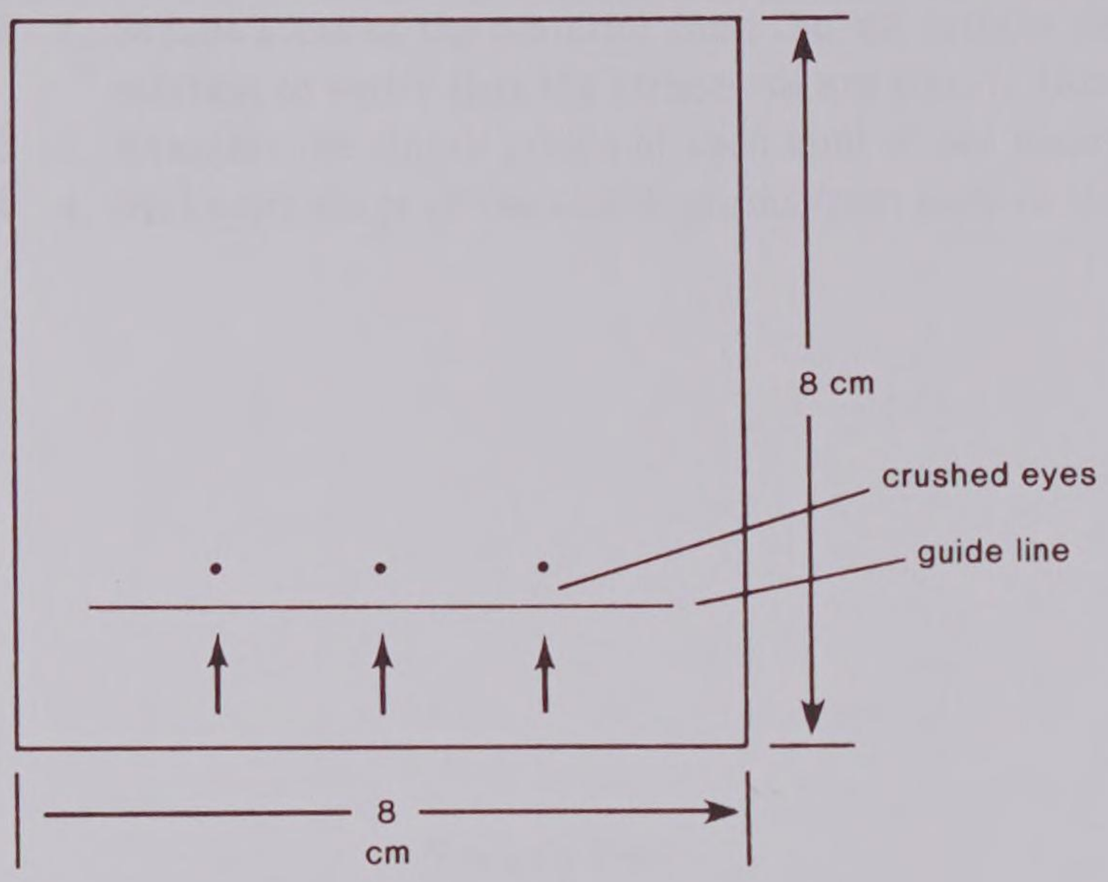
Drosophila Eye Pigments

Eye color in *Drosophila* is controlled by metabolic pathways that can most simply be represented as follows:

$$\text{Precursor (substrate)} \xrightarrow{\text{enzyme 1}} \text{Pigment A (substrate)} \xrightarrow{\text{enzyme 2}} \text{Pigment B (substrate)} \xrightarrow{\text{enzyme 3}} \text{Pigment C (end product)}$$

Mutant eye colors are caused by the fact that at least one of the enzymes present in the wild-type fly is inoperative in a mutant; this is due to the inheritance of a faulty DNA code. A *mutation* causes a change in the DNA code, which in turn causes an enzyme to have an abnormal sequence of amino acids. With this sequence of amino acids, the enzyme cannot function as it normally would. If an enzyme is nonfunctioning, its substrate accumulates. For example, if enzyme 2 were not functioning, what substrate would accumulate? _____

Figure 9.7 Placement of samples of chromatography paper. (From W. F. Andresen et al., *Laboratory Inquiries into Concepts of Biology.* © 1971 Kendall/Hunt Publishing Company, Dubuque, Iowa.)



In wild-type flies, all the various pigments of the metabolic pathway are present in the eyes. In mutant flies, some pigments are absent and others are present in increased amounts. It is possible to compare the pigments present in mutant eyes with those present in wild-type eyes by utilizing paper chromatography.

Experimental Procedure

S 1. Obtain a piece of chromatography paper 8 cm by 8 cm.
S 2. Draw 3 dots on a pencil line 2.4 cm from the bottom (fig. 9.7, use pencil only).
3. Make sure the dots are centered and at least 2.4 cm apart on the paper.
S 4. Label with a pencil the first dot "+" for wild-type, the second "r" for white eye, and the third "s" for sepia.
S 5. Remove the heads of 2–5 anesthetized wild-type flies of the same sex. Heads are removed quite simply by using a dissecting needle.
S 6. Place the heads on the dot and crush them with a clean glass rod.
S 7. Follow the same procedure for both white-eyed and sepia-eyed flies.
8. Describe the actual appearance of the eyes of each type of fly:

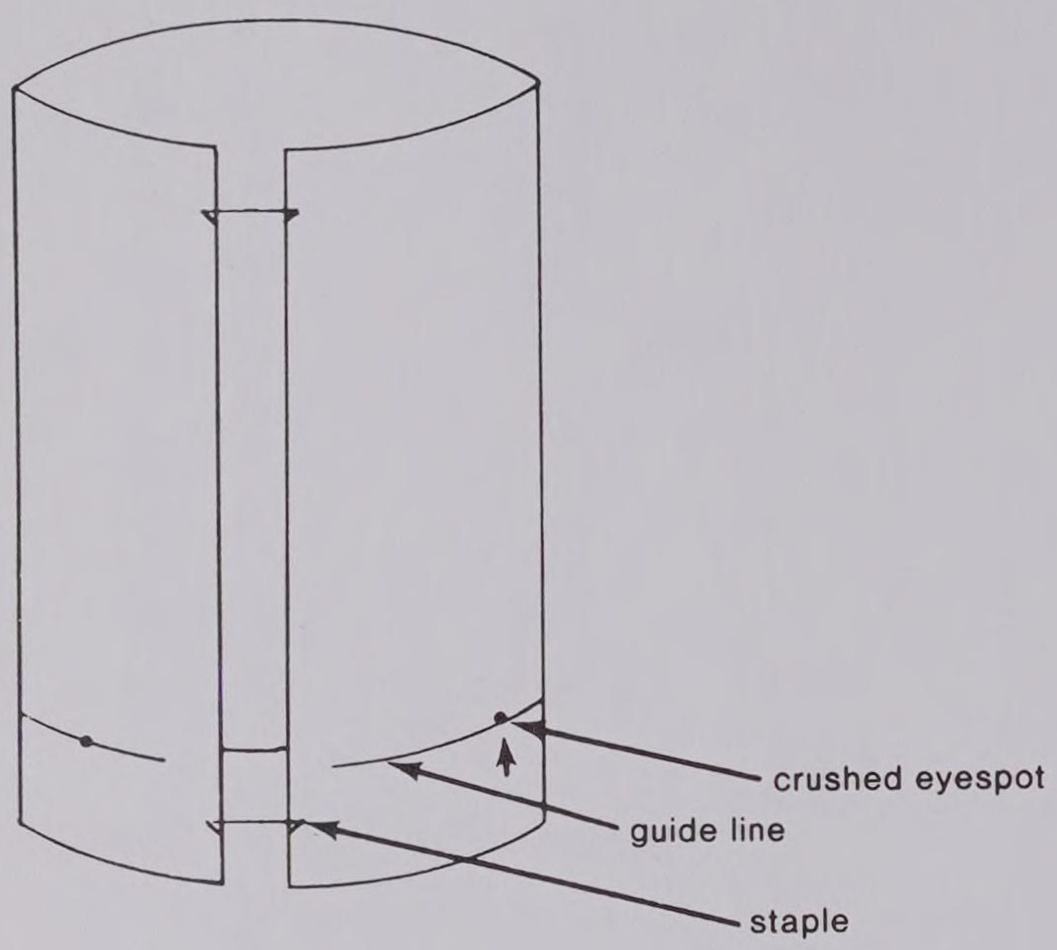
a. wild-type eye _____

b. white-eyed _____

c. sepia-eyed _____

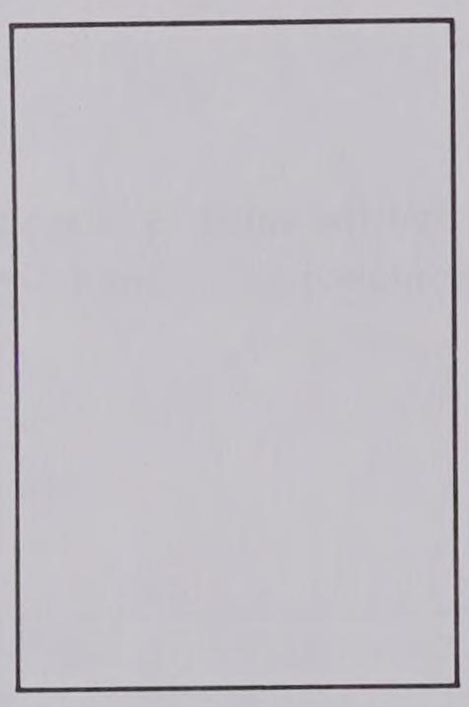
S 9. Fold the paper into a cylinder and attach the ends with staples (fig. 9.8).
S 10. Add 25 ml of *developing solution* into a darkened developing jar.
T 11. Place the paper in a darkened developing jar and allow to develop for 60 minutes or more. The longer the developing time, the better the pigment separation. The pigments are soluble in the solution to a different degree and will precipitate out on the paper when they reach the limit of their solubility.
S 12. After a developing period of 1 hour, remove the chromatogram and allow it to air dry (5 min) in the dark.
S 13. Turn off the room lights and examine with ultraviolet light (or in a UV view box). *Caution:* Never look at a UV light source, since the rays are *damaging* to the *retina.* Always keep the light bulb shaded. If the lamp tips over or falls on the floor, immediately close your eyes. A student wearing glasses can remove the cord from the outlet because UV rays do not penetrate glass to any extent.
S 14. Record your results for each chromatogram in the following diagrams. As an aid to doing so, circle the fluorescent areas with a pencil and reproduce this shape on the diagrams.

Figure 9.8 How to fold the chromatography paper. (From W. F. Andresen et al., *Laboratory Inquiries into Concepts of Biology.* © 1971 Kendall/Hunt Publishing Company, Dubuque, Iowa.)

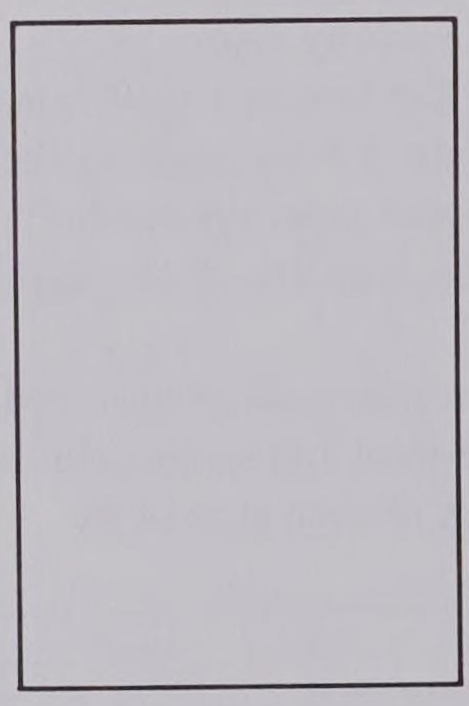


S 15. Write in on the diagrams the color of the various shapes. (If colored pencils are available, shade your diagrams with the appropriate color.)

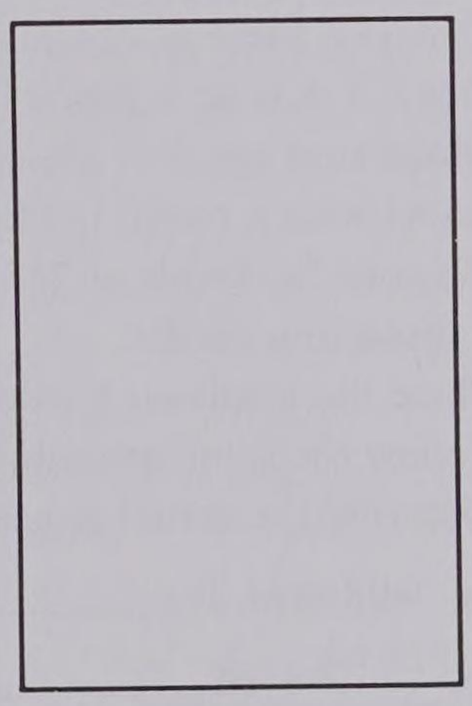
wild-type



white-eyed



sepia-eyed



Explain your results: _____

_____

_____

Starch Production in Peas

The activity of the starch-forming enzyme will be compared in smooth and wrinkled peas. The reaction to be studied is as follows:

$$\text{Glucose-1-phosphate} \xrightarrow{\text{enzyme}} \text{starch}$$

Starch Grains

1. Cut both a soaked smooth and a soaked wrinkled pea in half with a razor blade.
2. Scrape some of the material from the cut surface and use this to prepare a wet mount. Add some *iodine* solution to verify that the structures are starch. (Starch turns blue-black in contact with an iodine solution.)
3. Examine the starch grains of each kind of pea under the compound light microscope.
4. Make drawings of the starch grains from each of the seeds in the available space.

Smooth Pea

Wrinkled Pea

Enzyme Activity

1. Count out 10 of each type of presoaked peas.
2. Grind each sample of 10 peas, to which 10 ml of *water* has been added, in a clean mortar and pestle until there are no large pieces remaining.
3. Place the resulting liquid in labeled centrifuge tubes.
4. Centrifuge the samples for about 2 minutes to remove sediment. (Note: To save time, more than one team can centrifuge at the same time. *Be sure your tubes are properly labeled.*)
5. Place the resulting clear liquid, which contains a *starch-forming enzyme,* into separate (and labeled) test tubes.
6. Obtain a petri dish that contains *glucose-1-phosphate* agar. Using a wax pencil, divide the dish into right and left halves by drawing a line on the *bottom* side of the dish containing the agar.
7. Label and place on the right-hand side a drop of enzyme extract from the smooth peas.
8. Do the same on the left-hand side of the plate with the extract from the wrinkled peas. (Be sure the drops are well separated from each other.)
9. Allow these drops to remain undisturbed for 30 minutes.
10. At the end of 30 minutes, gently blot up the enzyme extract from the smooth peas and place a few drops of iodine solution where the drop of enzyme extract was. Repeat this procedure with the extract from the wrinkled peas.
11. Look for the appearance of a blue color in the agar around the edge of the enzyme drops, indicating starch formation. Which type pea was, when it was living (as evaluated by the presence of large starch grains), better able to synthesize starch from glucose-1-phosphate naturally available in the living plant? _____ Which type pea presently, in the ground-up form as a liquid in the glucose-1-phosphate agar petri dishes, has the greatest amount of starch-forming enzyme activity? _____
12. Record your results in table 9.1.

Table 9.1 Starch Grains and Enzyme Activity in Peas

	Starch Grains	Present Enzyme Activity (starch formation in petri dish)
Smooth peas		
Wrinkled peas		

Sickle-Cell Disease in Humans

Obtain a prepared slide of a normal blood smear and one showing sickle-cell disease (fig. 9.9). How do the slides differ?

_____

_____

One writer has described sickle-cell disease this way:

> The symptoms of sickle-cell anemia occur because the cells die off quickly and because the sickle-shaped cells clog the blood vessels. The cells have the sickle-shape because of the rodlike clumping of molecules within the cells. The molecules clump that way because in one tiny spot a chemical attaches on differently than it does in normal red blood cell molecules. The tiny chemical attaches that way because of the genes that were inherited from the person's ancestors.[1]

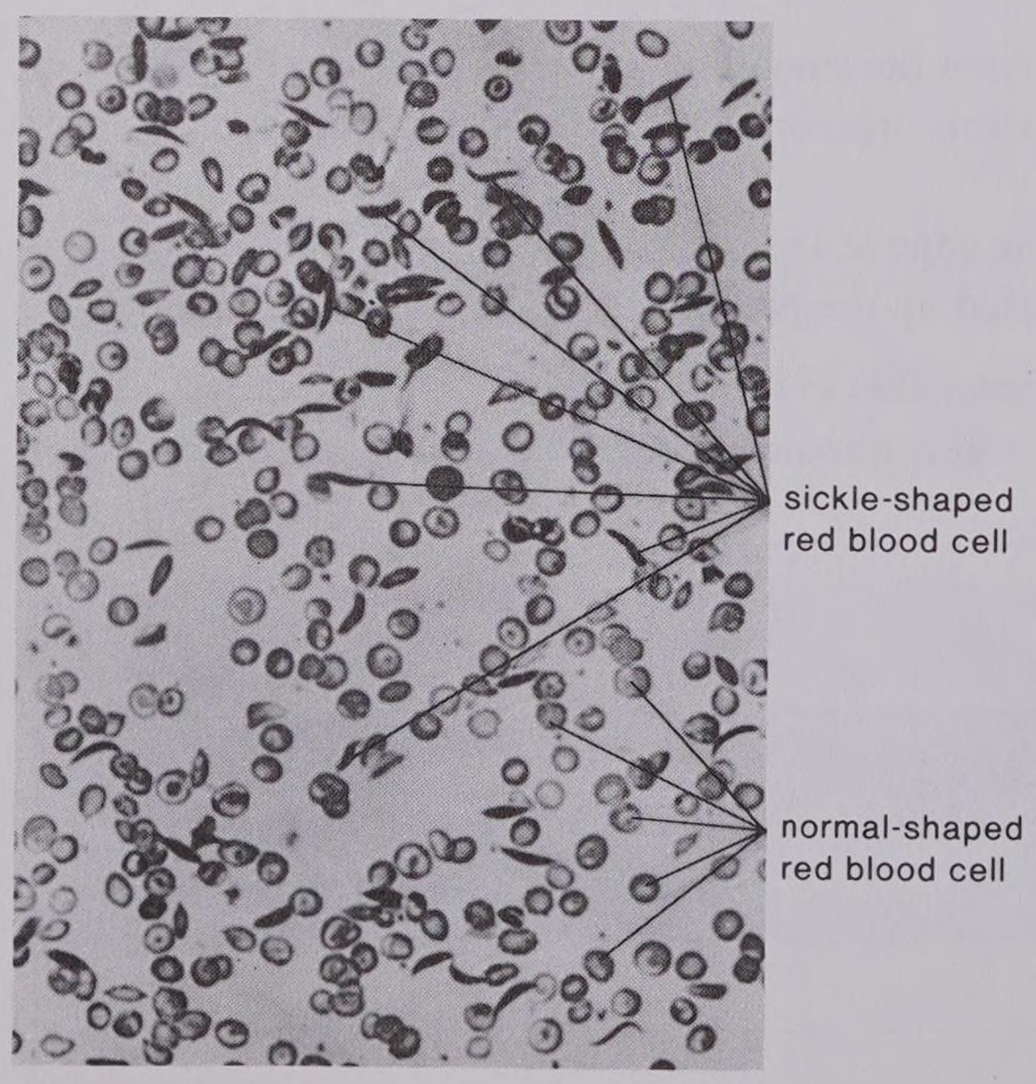
The molecule referred to in the paragraph is hemoglobin. The genotype for a normal person is Hb^AHb^A; for a person with sickle-cell disease it is Hb^SHb^S; and for a person with sickle-cell trait it is Hb^AHb^S. The tiny chemical referred to in the paragraph is an amino acid. According to figure 9.10 the amino acid _____ in normal hemoglobin has been changed to _____ in sickle-cell hemoglobin.

If CTT is the code for glutamic acid and CAG is the code for valine, then fill in table 9.2 to contrast sickle-cell disease with the normal condition.

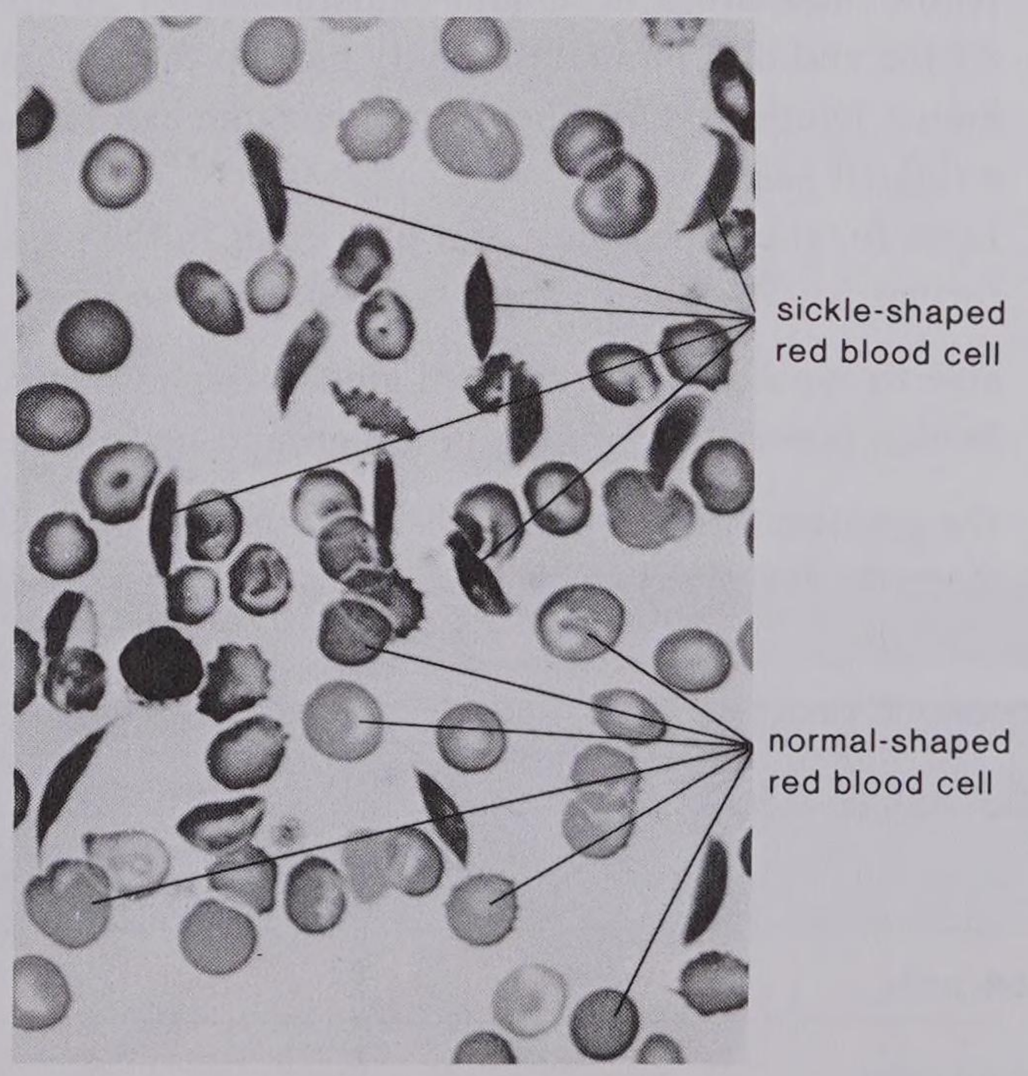
Table 9.2 Sickle-Cell versus Normal Hemoglobin

	DNA Code	mRNA Codon	Amino Acid
Sickle-cell			
Normal			

Figure 9.9 Sample of blood from individual with sickle-cell disease. ***a.*** Lesser power. ***b.*** Higher power. (Courtesy Carolina Biological Supply Company.)



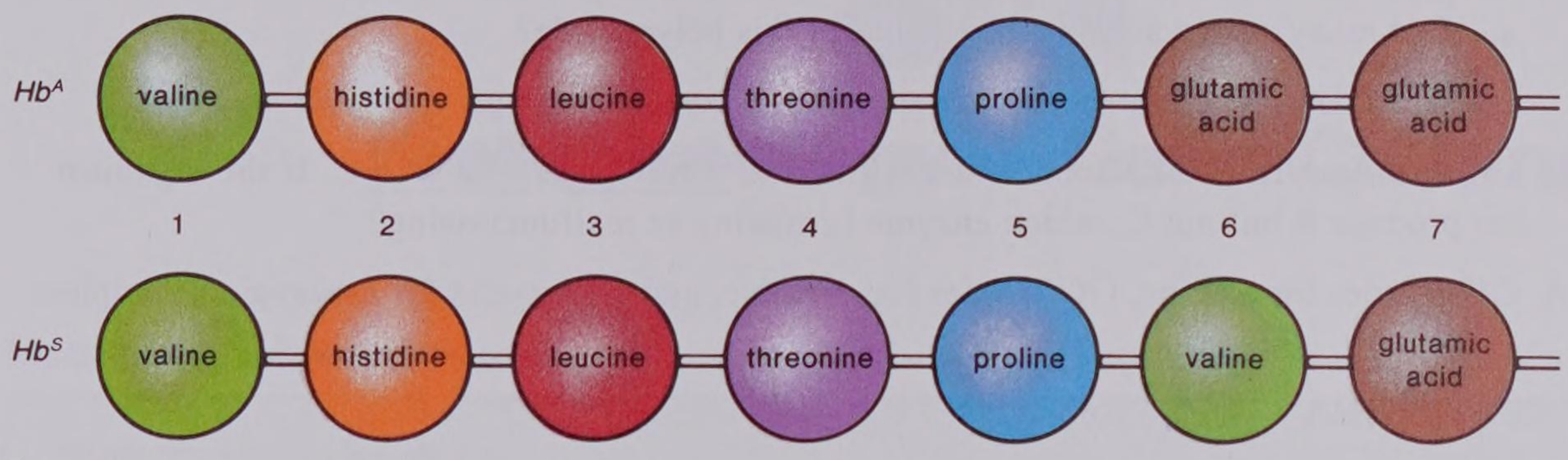
a.



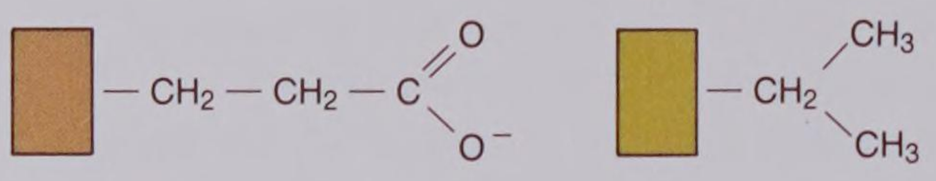
b.

[1]Linde, S. M. 1972. *Sickle Cell.* New York: Pavilion.

Figure 9.10 Normal hemoglobin versus sickle-cell hemoglobin. ***a.*** In the sixth position of the beta chain, sickle-cell hemoglobin has valine instead of glutamic acid. ***b.*** Glutamic acid has a polar R group, while valine has a nonpolar R group. This leads to a change in hemoglobin structure with resulting sickle cells.



a.



glutamic acid (polar R group)

valine (nonpolar R group)

b.

Laboratory Review 9

_____ 1. The gene that codes for a polypeptide contains 750 nucleotides.

_____ a. How many amino acids will be found in this polypeptide?

_____ b. How many tRNA molecules are required to construct the polypeptide?

_____ 2. An organism utilizes this metabolic pathway: A $\xrightarrow{\text{enzyme 1}}$ B $\xrightarrow{\text{enzyme 2}}$ C. If the organism can produce B but not C, which enzyme is missing or malfunctioning?

3. Knowing that in DNA, CGA codes for alanine, GCA codes for arginine, and TTA codes for asparagine, complete the following:

DNA	mRNA	tRNA	Amino acid
C			
G			
A	_____	_____	_____
G			
C			
A	_____	_____	_____
T			
T			
A	_____	_____	_____

4. What is the relationship between phenotype and genotype?

5. What happens during transcription and translation?

6. What is a codon and what does it do?

References

Andresen et al., 1971. *Laboratory Inquiries into Concepts of Biology.* Dubuque, IA: Kendall/Hunt Publishing Company.

Bonaudi, W. C., and Hoffman, E. A. 1970. *Laboratory Biology.* Dubuque, IA: Kendall/Hunt Publishing Company.

Snow, B. L., ed. *Biological Laboratory Exercises.* Boston, MA: Suffolk University.

Laboratory

10

Biotechnology—Gene Cloning

Learning Objectives

Students should be able to

1. explain the role of the plasmid in recombinant DNA technology;
2. discuss the function of a restriction enzyme;
3. describe the nature of recombinant DNA and the transformed cell within which it resides;
4. explain the function of cloning a transformed cell.

Introduction

This laboratory introduces you to basic techniques of *biotechnology gene cloning*. During the gene cloning process, you will work with a normal (uncut) plasmid, a restricted (cut) plasmid, and a ligated (rejoined) plasmid. These DNA samples will be used for gene cloning and may also be subjected to electrophoresis in order to compare their chemical makeup (Laboratory 11).

In recent years a biotechnology industry has grown out of recombinant DNA techniques. Certain bacteria, such as *E. coli,* have rings of extrachromosomal DNA called *plasmids* (fig. 10.1). These plasmids very often contain a gene that makes bacteria resistant to an antibiotic. For example, they may contain a gene that makes bacteria resistant to ampicillin. Plasmids can be extracted from bacteria and then enzymatically sliced into fragments using *restriction enzymes*. After this is done, a foreign gene can be attached to these fragments before the plasmids are enzymatically rejoined using a *ligase enzyme*. The plasmids now contain recombined, or *recombinant, DNA* molecules, and such plasmids will be taken up by other bacterial cells. Once a plasmid has entered a bacterial cell, the cell is said to be *transformed* because it can now make a protein product it was unable to make before. It is possible to detect transformed bacteria because they will be resistant to an antibiotic (e.g., ampicillin). Whenever a transformed bacterium reproduces, the plasmid, including the foreign gene, is copied. Since exact multiple copies of the plasmid and gene are now available, they are said to have been *cloned*.

The biotechnology industry uses gene cloning to produce unlimited quantities of a protein product. For example, gene cloning allows insulin and growth hormone to be inexpensively produced today.

In this laboratory, you have the opportunity to work with the recombinant DNA techniques of restriction, ligation, transformation, and cloning (fig. 10.2); however, we will not be inserting a foreign gene into the DNA. We will simply fragment the provided plasmids and anneal (rejoin) them once again. The plasmids are ones that contain a gene that makes bacteria resistant to the antibiotic ampicillin. Bacteria that are not transformed will be sensitive to this antibiotic, while those that are transformed will be resistant to this antibiotic.

Specifically, we will be doing the following procedures:

1. Restriction. Plasmid DNA will be sliced with a restriction enzyme.
2. Ligation. The DNA fragments will be annealed (rejoined) with a ligase enzyme.
3. Transformation and cloning. Competent *E. coli* are strains that display an ability to take up plasmids. Even so, typically, very few bacteria become transformed. Transformed bacteria will produce colonies on nutrient agar plates that contain ampicillin.

Figure 10.1 Gene cloning is accomplished by using either recombined plasmid DNA or recombined viral DNA. A photomicrograph of the workhorse of biotechnology, the bacterium *E. coli.*

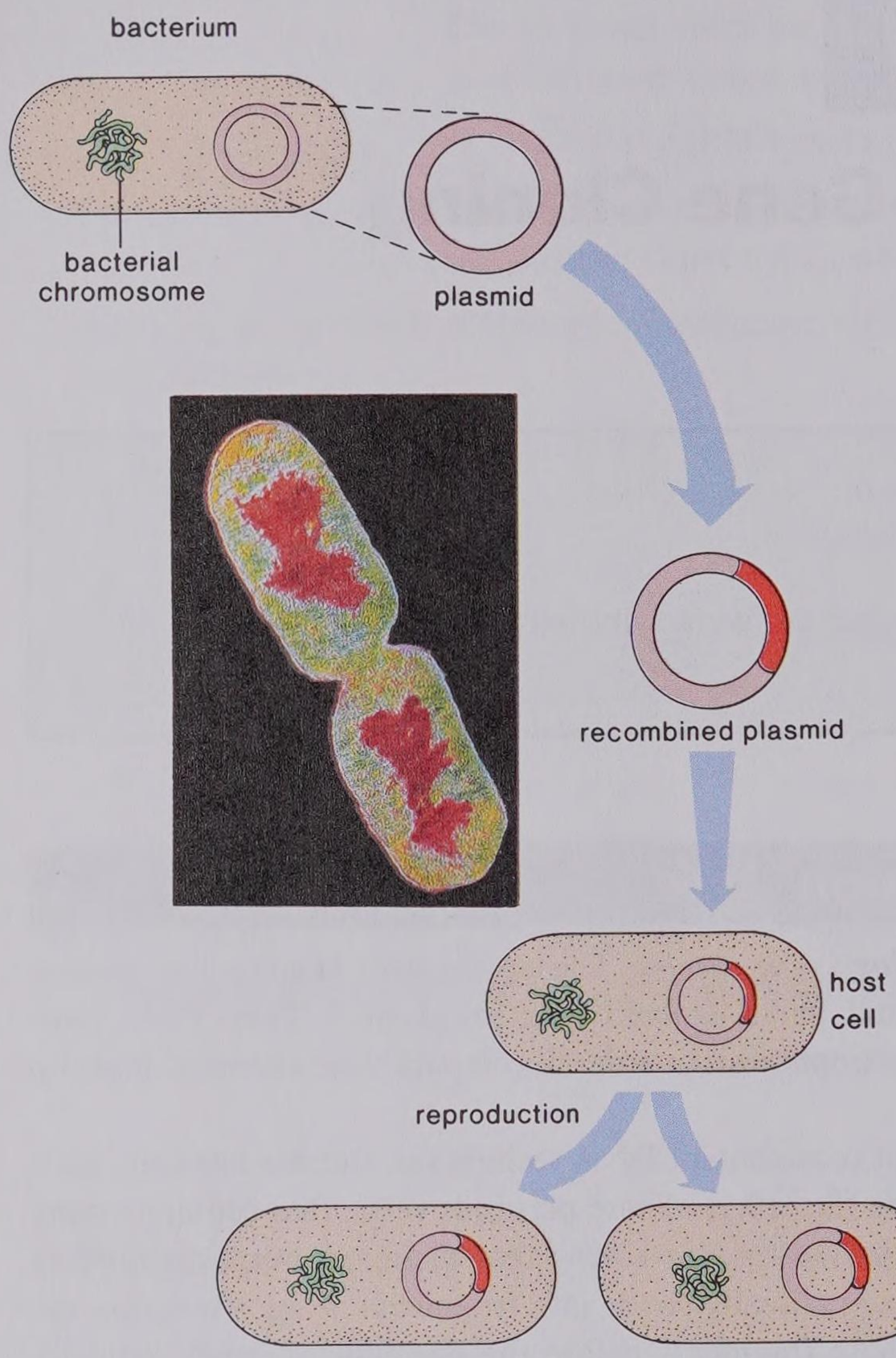
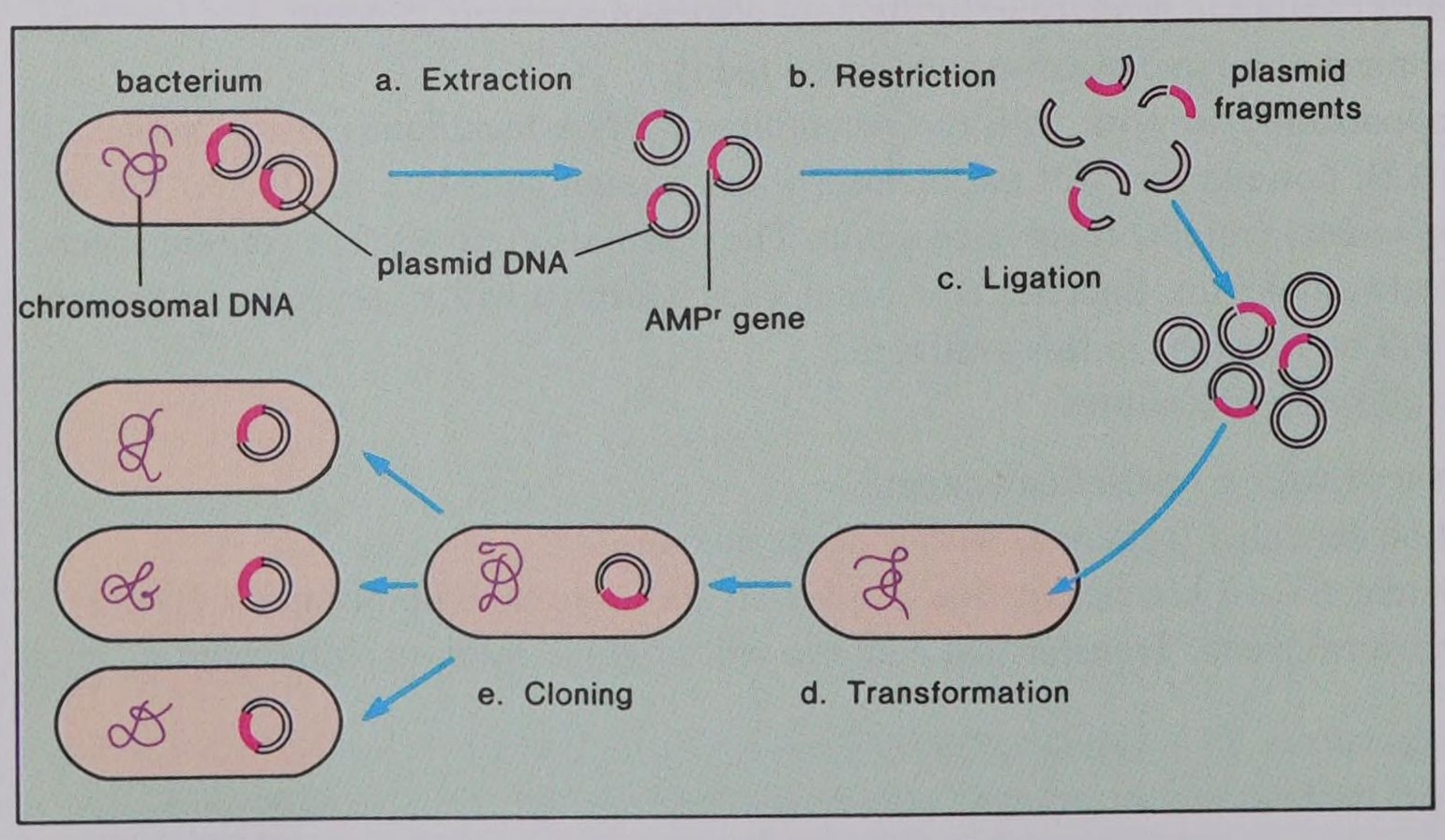


Figure 10.2 Basic laboratory methods. ***a.*** *Extraction:* Plasmids containing ampicillin resistance genes are extracted from *E. coli* bacteria. ***b.*** *Restriction:* Plasmids are "cut" into fragments by restriction enzymes. (At this point researchers could add foreign gene.) ***c.*** *Ligation:* Plasmids are ligated (annealed) with DNA ligase. ***d.*** *Transformation:* Plasmids are inserted into bacterial cells that do not contain the antibiotic resistance gene. ***e.*** *Cloning:* Transformed cells are inoculated into a growth medium containing the antibiotic ampicillin. Those bacteria containing the ampicillin resistance gene will survive and multiply.



Pipetting Technique

Before beginning the experiment, it is essential that you learn to pipette accurately. You will need a micropipetter, pipette tip, and a beaker of water.

1. Take a pipetter in your hand and gently depress the top until you feel a resistance. This is the *stop* point. Continue to depress the top of the pipetter until it will not move any farther. This is the *empty* point.
2. Fit a pipette tip onto the end of the pipetter.
3. Depress the top of the pipetter to the *stop* point, and put the pipette tip into a beaker of water. Release the top of the pipetter. A quantity of water will be drawn into the pipette tip.
4. Raise the micropipetter and its tip from the surface of the water.
5. Depress the top of the micropipetter to *empty*. A small drop will form at the end of the pipette tip.
6. Gently touch this drop to the side of the beaker. Do this several times with both a 5 μl and 100 μl pipetter.
7. Your instructor may also require that you check your accuracy by a method he or she will describe.

Restriction Procedure

1. With a marking pencil, label a 1.5 ml tube with an A. This is *tube A*.
2. Add to this tube the following:
   a. 5 μl *plasmid DNA*. (Retain rest of plasmid DNA. You will need it later.)
   b. 5 μl *restriction enzyme*.
3. Mix by tapping; spin gently if centrifuge is available.
4. Incubate at 37° C for 25 minutes.
5. Place in 65° C water bath for 10 minutes.
6. Complete all of the previous steps prior to starting the ligation procedure.

Ligation Procedure

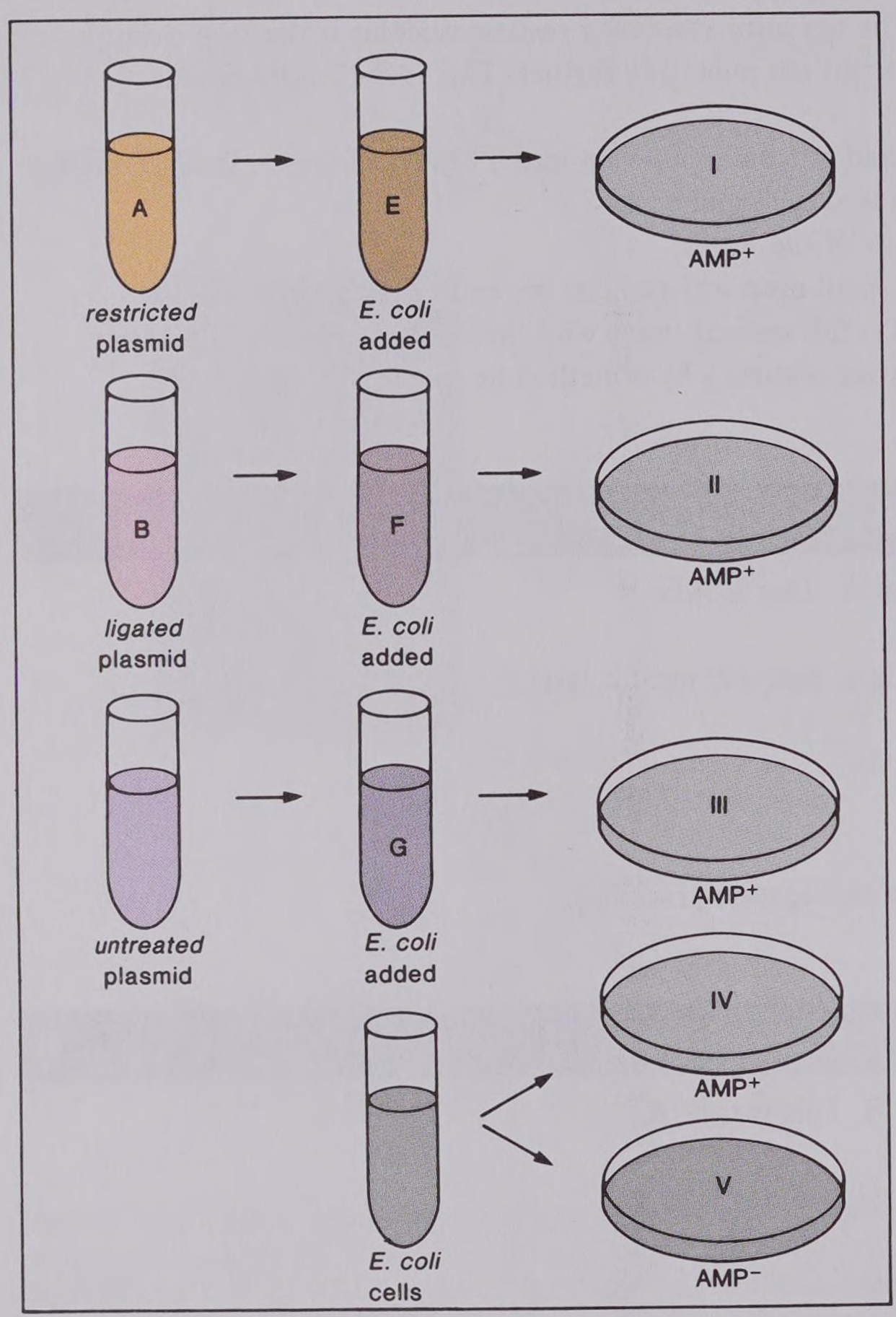
1. With a marking pencil, label a 1.5 ml tube with a B. This is *tube B*.
2. Add to this tube the following:
   a. 5 μl from tube A. (Retain rest of tube A. You will need it later.)
   b. 5 μl *ligase enzyme*.
3. Mix by tapping; spin gently if centrifuge is available.
4. Incubate at about 17° C (cool room temperature is also acceptable) for 1 hour.
5. Complete all of the previous steps prior to starting the transformation and cloning procedure.

Transformation and Cloning Procedure

1. Refer to the flow diagram in figure 10.3.
2. Obtain 4 nutrient agar AMP^+ plates and label them I, II, III, and IV.
3. Obtain one nutrient agar AMP^- plate and label it V.
4. Have available: *E. coli,* tube B, tube A, and plasmid DNA. Mix *E. coli* to ensure even distribution of cells.
5. Place tube B in 65° C water bath for 10 minutes; then cool to room temperature.

S 6. Obtain and label 3 microcentrifuge tubes as E, F, and G. To each of these tubes add 100 μl competent *E. coli* cells.

Figure 10.3 Flow diagram for cloning experiment.



S a. To tube E, also add 5 μl from tube A, using a clean pipette tip. Mix. This tube now contains *E. coli* plus *restricted* DNA.

S b. To tube F, also add 5 μl from tube B, using a clean pipette tip. Mix. This tube now contains *E. coli* plus *ligated* DNA.

S c. To tube G, also add 5 μl untreated plasmid DNA using a clean pipette tip. Mix. This tube now contains *E. coli* plus *untreated* plasmid DNA.

I 7. Place tubes E, F, and G in *ice* bath for 20 minutes.

I 8. Transfer these tubes to 42° C water bath for 90 seconds (heat pulse) and then to 37° C water bath for 2 minutes.

S 9. Using a clean pipette tip and a spreader sterilized according to the method described by your instructor:
   a. Transfer 100 μl of tube E to plate I, then spread.
   b. Transfer 100 μl of tube F to plate II, then spread.
   c. Transfer 100 μl of tube G to plate III, then spread.
   d. Transfer 100 μl of competent *E. coli cells* to plate IV and to plate V.

I 10. Place plates in 37° C incubator (upside down) for overnight growth.

Results

S Observe your plates and record the number of colonies of *E. coli* bacteria in table 10.1.

Table 10.1 Cloning Experiment

Plates	Type of Agar	Type of Plasmid	Number of Colonies
I			
II			
III			
IV			
V			

Analysis

1. Is plate V covered with bacteria? _____ Why would this be expected? _____

2. Does plate IV have no bacterial colonies? _____ Why would this be expected? _____

3. Of the 3 remaining plates, does plate I have the least amount of colonies? _____ Why would this be expected? _____

4. Does plate II have more colonies than plate I? _____ Why would this be expected? _____

5. Does plate III have more colonies than plate II? _____ Why would this be expected? _____

6. What were sources of error in your experiment? _____

7. How do your results compare with those of your classmates? _____

Laboratory Review 10

1. What do restriction enzymes do?

2. What do ligation enzymes do?

3. What is transformation?

4. What is gene cloning?

5. What does recombinant DNA mean?

Reference

Myers, R. M. Recombinant DNA technology in high school biology laboratory. *American Biology Teacher,* January 1988, 43.

Laboratory

11

Biotechnology—Gel Electrophoresis

Learning Objectives

Students should be able to

1. explain the general principles behind the separation of large organic molecules by electrophoresis;
2. describe the function that the electrophoresis buffer and agarose gel serve in these experiments;
3. evaluate the results of these experiments.

Introduction

This laboratory introduces you to a basic technique of biotechnology, gel electrophoresis. Gel electrophoresis is used quite often to study biochemical differences between macromolecules such as proteins and DNA.

Pipetting Technique

Before beginning the experiment, it is essential that you learn to pipette accurately. If necessary read page 117 in Laboratory 10 and practice this technique.

Gel Electrophoresis

The 2 most widely used techniques for separating molecules in biotechnology are chromatography and gel electrophoresis. Chromatography works best for separating small molecules and processing large batches. *Electrophoresis,* which works best to separate macromolecules such as DNA fragments and proteins, is a part of this laboratory.

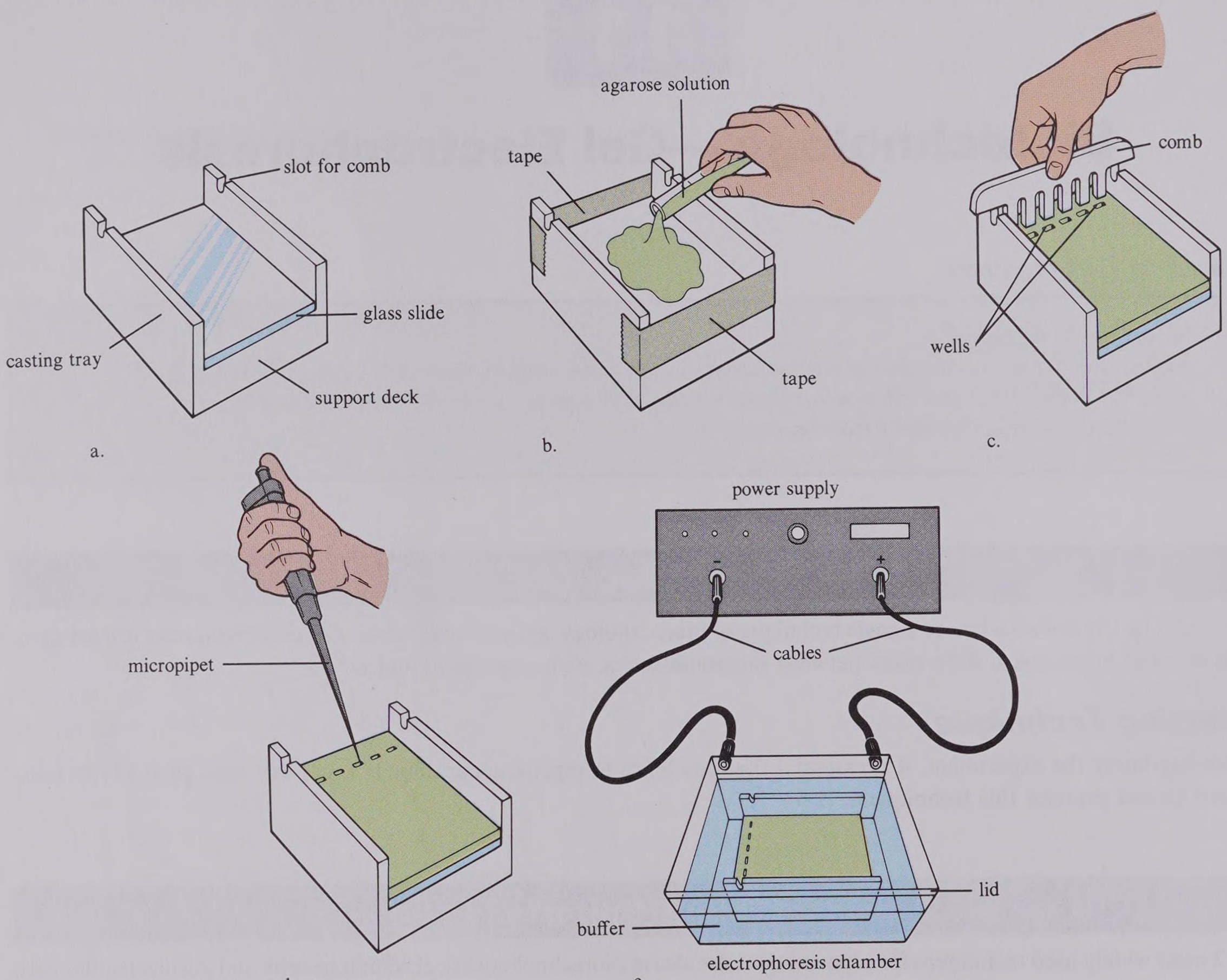
Gel electrophoresis has many applications. It has been used to distinguish cancer types, to test for genetic disorders, to assess an individual's risk of coronary disease, and to determine the nucleotide sequences in many organisms, including humans.

During gel electrophoresis, charged macromolecules migrate across a span of gel because they are placed in an electrical field. First, the gel acts like a sieve to retard the passage of molecules according to their size and shape. Second, the molecules that are charged negatively will migrate toward the positive electrode, while those that are charged positively will migrate toward the negative electrode.

While many designs of electrophoresis equipment are possible, almost all gel electrophoresis is carried out with horizontal gel slabs because such gels are very simple to prepare and load. First, the gel is poured onto a glass plate and wells are formed. The plate is installed on a platform in an electrophoresis chamber and buffer is added. Samples are loaded into the wells[1] and then the electrical current, which causes the molecules to migrate, is turned on. After staining, the various macromolecules appear as a series of bands spread from one end of the gel to the other (fig. 11.1).

[1]The wells can also be loaded before adding the buffer. Then, they will need to be sealed with agarose solution.

Figure 11.1 Equipment and procedure for gel electrophoresis. ***a.*** Casting tray for making gel slab. ***b.*** Pouring agarose solution into tray. ***c.*** Removing the comb. ***d.*** Loading the wells. ***e.*** Electrophoresis chamber and power supply.



Preparing the Gel Slab

It is possible to purchase precast gel slabs. You also can prepare your own, as outlined next.

1. Prepare a mold for your gel solution. This can be done by simply taping all edges of a glass slide. Or, if available, place the glass slide on the support deck of a casting tray and tape the ends of the support deck securely (fig. 11.1*a*).
2. Add the amount of *agarose* powder directed by your instructor to a measured quantity of *electrophoresis buffer* in a test tube.
3. Heat the test tube in a boiling *water bath* until the agarose dissolves. After boiling for about 1 minute, remove the test tube and let it cool for 2–3 minutes.
4. Pour the warm agarose solution into the mold (casting tray) (fig. 11.1*b*).
5. Immediately insert a comb into the gel, pushing down gently. The teeth of the comb should come to rest about 0.5 mm–1.0 mm above the glass slide. The comb will form wells to receive your samples (fig. 11.1*c*).
6. After the gel is completely set (about 15 min), carefully remove the strips of tape and the comb by lifting it straight up and away from the gel. You now have a line of wells that will be filled with macromolecule samples (fig. 11.1*d*).

Electrophoresis

S 1. Transfer the gel (in the casting tray if so directed) to the electrophoresis chamber. Usually several gels can be placed in the chamber at the same time. Position the gels so that the wells are closest to the negative electrode.

S 2. Slowly add just enough *electrophoresis buffer* to cover the gel to a depth of 1 mm–3 mm. The experiment in the "Separation of Protein Samples" section does not require the addition of ethidium bromide. Ethidium bromide may be dissolved in the electrophoresis buffer to make DNA visible under ultraviolet illumination upon completion of electrophoresis. If *ethidium bromide* has been added to the electrophoresis buffer in order to stain DNA fragments, be careful *not* to let the solution *touch* the *skin* since ethidium bromide is a mutagen and possible carcinogen. Wear rubber gloves when working with an electrophoresis solution that contains ethidium bromide.

S 3. Each macromolecule sample is in a buffer (called a sample buffer) and, if it is not visibly colored, it contains a tracking dye. Each student will load a number of wells as directed by the instructor, using a micropipet (see page 117) as in Laboratory 10. *Dispense* 10 μl–15 μl of each sample into a different well (fig. 11.1*d*). As before, the micropipet can be rinsed between samples by drawing and expelling distilled water 3 times.

T 4. Close the lid of the electrophoresis chamber. Connect the cables with the power supply off; when ready, turn on the power supply (fig. 11.1*e*) when approved to do so by your instructor.

S 5. Be sure to *terminate electrophoresis* before the foremost pigment (or tracking dye) has reached within *¼ cm* of the positive electrode end of the gel.

S 6. Remove your gel slab (or casting tray) and analyze your results. Measure the distance of each band from the sample wells.

Separation of Protein Samples

S Load the wells with 10 μl of each of these samples, which are already in sample buffer. These proteins, except for serum albumin, are pigments and therefore do not need a tracking dye. A blue tracking dye has been added to the sample buffer for albumin.

Cytochrome c: This is one of the cytochromes in the respiratory chain found in mitochondria. The molecule consists of a polypeptide chain and a heme group. It is the iron-containing heme group that gives the molecule an orange-brown color.

Myoglobin: Myoglobin, like hemoglobin, is a red respiratory pigment that also has an iron-containing heme group. Myoglobin binds to and stores oxygen in muscles.

Phycocyanin: This is the pigment that gives a blue-green color to cyanobacteria. It helps absorb light energy and assists these organisms in carrying on photosynthesis.

Serum albumin: This molecule is found in blood, where it transports many smaller molecules. Because it is not naturally colored, a blue tracking dye has been added to the sample buffer.

Results

At the completion of electrophoresis (or as soon as 6 minutes after the start), you will see various bands in each lane.

S Measure the distance in cm of each band from the sample wells and complete table 11.1.

Table 11.1 Electrophoresis of Protein Samples

Sample	Distance Migrated (cm)
Cytochrome c	
Myoglobin	
Phycocyanin	
Serum albumin	

Table 11.2 Chemical Properties of Protein Samples

Sample	Isoelectric Point	Charge (pH 8.6)	Charge (pH 6.0)
Cytochrome c	10.2		
Myoglobin	7.2		
Phycocyanin	6.5		
Serum albumin	4.8		

Analysis

Proteins differ from one another not only in size but also in charge. The charge differences of proteins at the same pH are largely due to the differences in their R groups. pH affects the charge of proteins because it affects whether or not groups like amino (NH_2) and carboxyl (COOH) groups ionize. Amino groups become NH_3 in acidic solutions, and carboxyl groups become COO^- in basic solutions.

Each protein has an *isoelectric point,* the pH at which they are *not charged* at all. In *pH* solutions *higher* than the isoelectric point, a protein becomes more and more *negatively* charged. In *pH* solutions *lower* than the isoelectric point, a protein becomes more and more *positively* charged. The isoelectric points of the proteins you submitted to electrophoresis are given in table 11.2.

Ⓢ Complete the column in the table that indicates the expected charge of each protein at pH 8.6, the pH of the electrophoresis buffer used in your experiment.

Does this information help explain your results? _____ Why? _____

_____

Ⓢ Complete the last column in table 11.2 to indicate the expected charge for each protein at pH 6.

What results would you have expected at this pH? _____

_____

Separation of Plasmid DNA Samples (Optional)

This experiment is a continuation of the gene cloning experiment that began on page 117 in Laboratory 10.

Ⓢ 1. Obtain and label 3 microcentrifuge tubes H, I, and J. To each of these tubes add 5 μl of *sample buffer.*
   a. To tube H, also add 5 μl of *untreated plasmid DNA.*
   b. To tube I, also add 5 μl from tube A (*restricted plasmid DNA*).
   c. To tube J, also add 5 μl from tube B (*ligated plasmid DNA*).

Ⓢ 2. Cast a gel slab and prepare an electrophoresis chamber as directed.

Ⓢ 3. Load the wells with 10 μl from each of the 3 tubes.

Ⓣ 4. Conduct electrophoresis as previously directed.

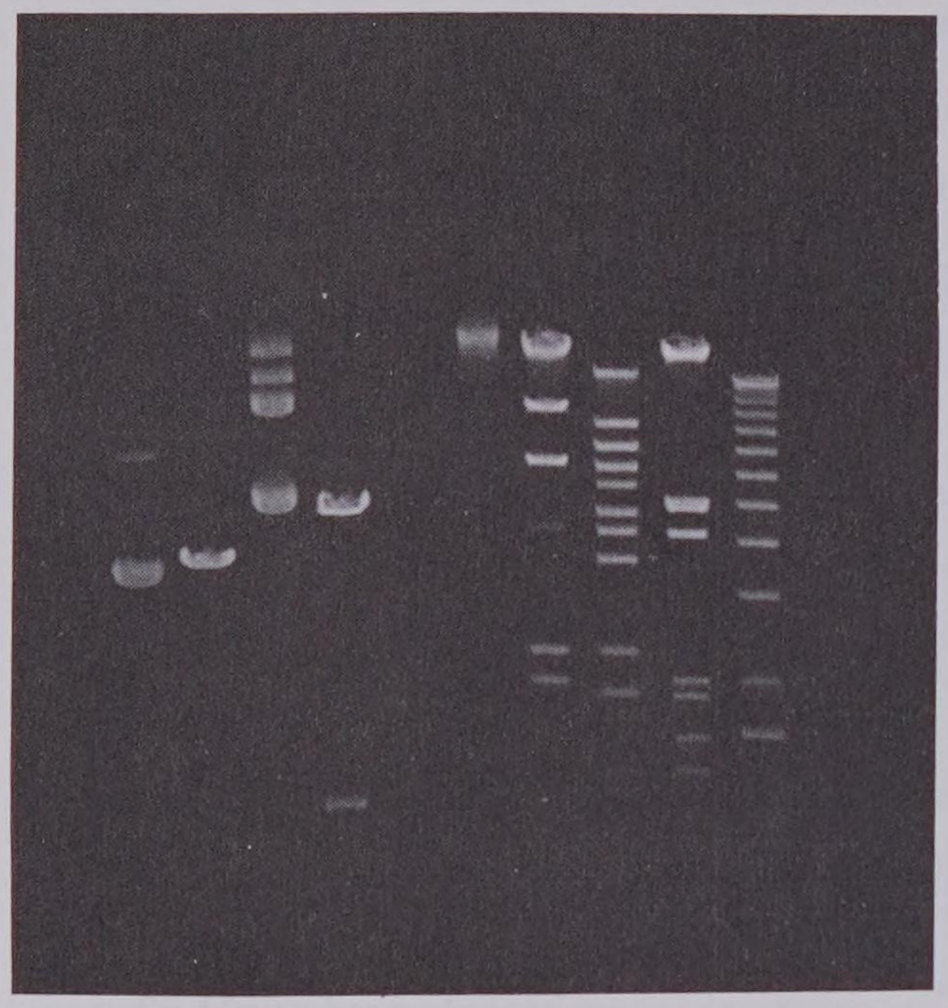
Results

Ⓢ 1. Using rubber gloves, transfer the gel to an ultraviolet light chamber for viewing and documentation. (*Caution:* Ultraviolet is damaging to the retina. *Do not look into the light.*)

Ⓢ 2. Ask your instructor if you should obtain a camera to photograph the results of your electrophoresis so that there is a permanent record of the results.

Ethidium bromide, which has been added to the electrophoresis buffer, stains the DNA so that it can be seen under ultraviolet light. It is very important *not* to allow the electrophoresis buffer to come in *contact* with the *skin* since ethidium bromide is a *mutagen* and possible *carcinogen.* Methylene blue staining is an alternative to the use of ethidium bromide.

Figure 11.2 Sample electrophoresis results. The samples contain different macromolecules that have separated out.



3. Examine your electrophoresis photograph and measure the distance in centimeters of each band from the original samples. (Measurements can be taken directly from the gel slab if photographic equipment is not available.)
4. Complete table 11.3, and compare to figure 11.2.

Table 11.3 Electrophoresis of Plasmid DNA Samples

Sample	Distance Migrated (cm)
"Uncut" plasmid DNA (tube H)	Band 1:
	Band 2:
	Band 3:
DNA digest (tube I)	Band 1:
	Band 2:
	Band 3:
Ligated DNA (tube J)	Band 1:
	Band 2:
	Band 3:

Analysis

Why would you expect your "uncut" plasmid DNA (tube H) sample to only have one band? _____

_____

Why might your restricted plasmid sample (tube I) show different degrees of migration? _____

_____

Why might your ligated plasmid sample (tube J) show different degrees of migration? _____

_____

Laboratory Review 11

_____ 1. What type of enzyme anneals DNA plasmids that have been cut?

_____ 2. What term is used to describe bacteria that have taken up a plasmid?

_____ 3. After cloning has occurred, there are how many copies of the same type of plasmid or gene?

_____ 4. A plasmid that is carrying foreign genes is called what type of DNA?

_____ 5. Transformed bacteria in this laboratory (can, cannot) grow on nutrient agar containing ampicillin.

_____ 6. During electrophoresis, macromolecules are separated according to what 2 characteristics?

_____

_____ 7. What do you call the piece of equipment that makes the wells on an agarose gel slab?

_____ 8. If a sample contains 2 DNA molecules of different lengths, how many bands will there be for this gel lane?

_____ 9. A protein with an isoelectric point of 5 is (negatively or positively) charged at pH 6?

_____ 10. The protein in question 9 will migrate toward which electrode?

References

Davis, L. G., Dibner, M. D., and Battey, J. F. 1986. *Basic methods in molecular biology.* New York: Elsevier Science Publishing Co.

Maniatis, T., Fritsch, E. F., and Sambrook, J. 1982. *Molecular cloning: A laboratory manual.* New York: Cold Spring Harbor Laboratory.

Micklos, D., and Greyer, G. 1990. *DNA Science: A First Course in Recombinant DNA Technology.* Available through Carolina Biological Supply Co.

Myers, R. M. Recombinant DNA technology in high school biology laboratory. *American Biology Teacher,* January 1988, 43.

Films and Videos

The Instructor's Manual for *Biology* contains suggestions for films that could be shown during this laboratory.

Laboratory

12

Evidence of Evolution

Learning Objectives

Students should be able to

1. define and recognize possible fossils;
2. describe in general terms the time scale of evolution;
3. explain how embryology offers proof of evolution;
4. compare the human skeleton with the monkey skeleton in at least 8 ways;
5. explain how biochemistry is an aid to the study of the evolutionary relationship between organisms.

Introduction

The theory of *evolution* states that life began with the first cell(s) and that all organisms have evolved from this (these) cell(s). It is not agreed upon by scientists as to whether evolution occurred at a steady, gradual rate or in spurts (punctuated equilibrium). Evidence in support of evolution, then, must show that organisms have changed over the ages but that the most closely related still have many features in common.

This laboratory will study 4 types of data in support of evolution: (1) fossil evidence, (2) embryological comparison, (3) comparative anatomy, and (4) biochemical comparison.

Fossil Evidence

Fossils are the remains or evidence of some animal or plant that lived long ago. The formation and preservation of fossils require that the animal or plant part be protected against destruction by weather or decomposing organisms. There are several well-known examples where such conditions have occurred. Artifacts from the Swiss lake dwellers of hundreds of years ago have been found at the bottom of lakes. Mummified humans have been preserved in bogs in Denmark because the acidity of the peat prevented decomposition. Other places where fossils are abundantly found include quicksand and asphalt pits such as the famous La Brea tar pits near Los Angeles. These tar pits contain thousands of animals that became trapped and entombed in their depths. Briefly, fossils are formed most commonly by the following 3 methods.

Petrifaction occurs when the original hard part and, less frequently, the soft parts are replaced by minerals. The result is that a bone is no longer as it was in the animal but is of an entirely different chemical composition. One example of this process is petrified wood, which is no longer wood but stone.

A *mold* is formed by the hardening of material surrounding a buried organism, followed by the decay and "removal" of the organism by groundwater.

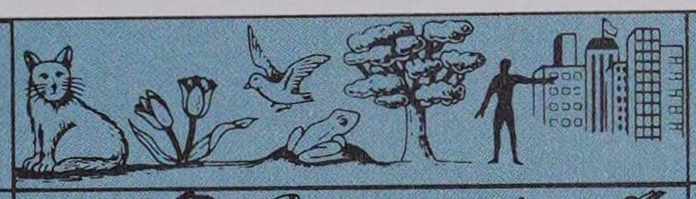
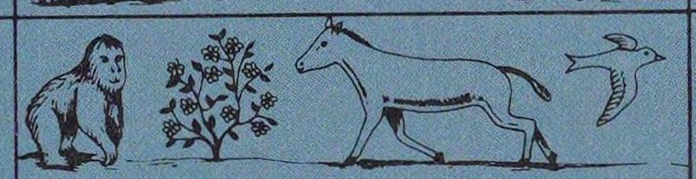
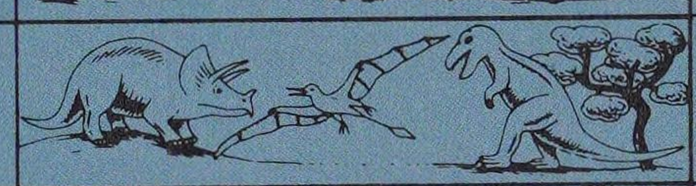
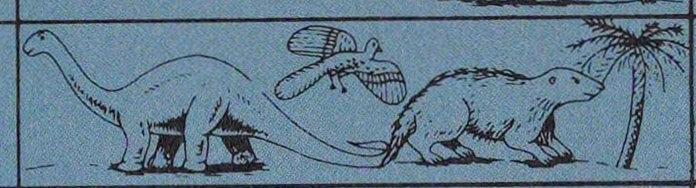
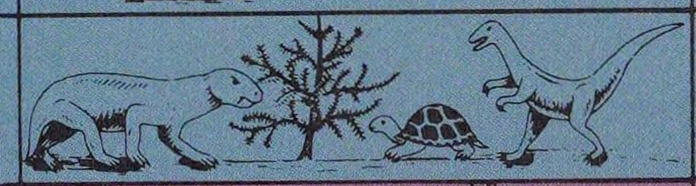
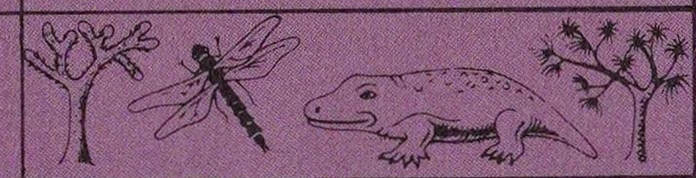
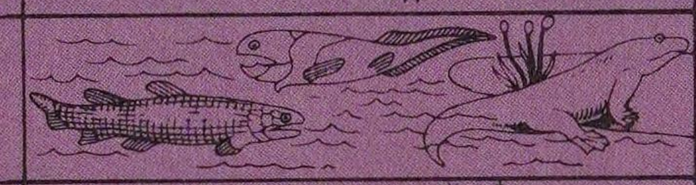
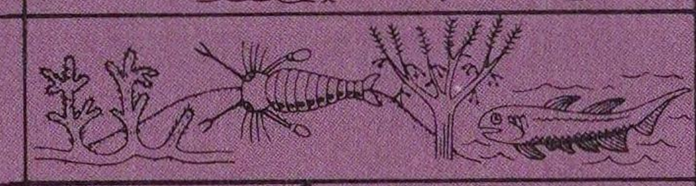
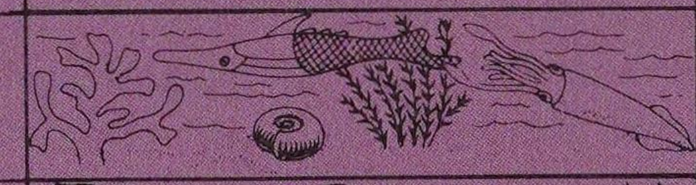
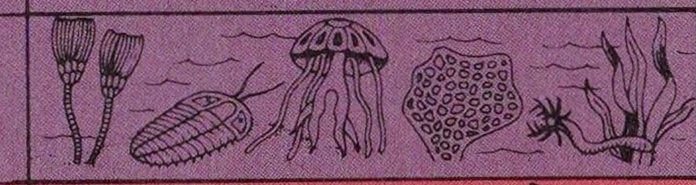
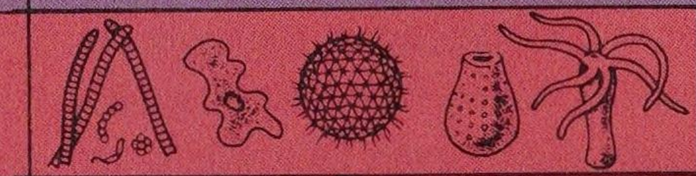
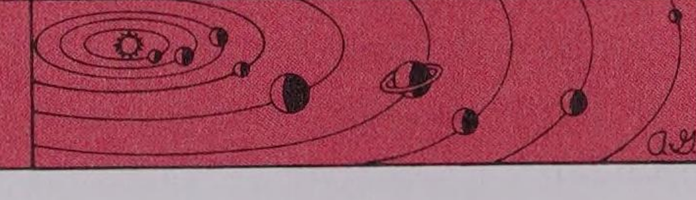
A *cast* can be a continuation of the mold-making process if, once the organism has been "removed" to form a mold, minerals fill the space previously occupied by the organism or component structure and, upon hardening, form a replica of the original.

1. Using figure 12.1 answer the following questions:

   Name the epoch of the Ice Age. _____

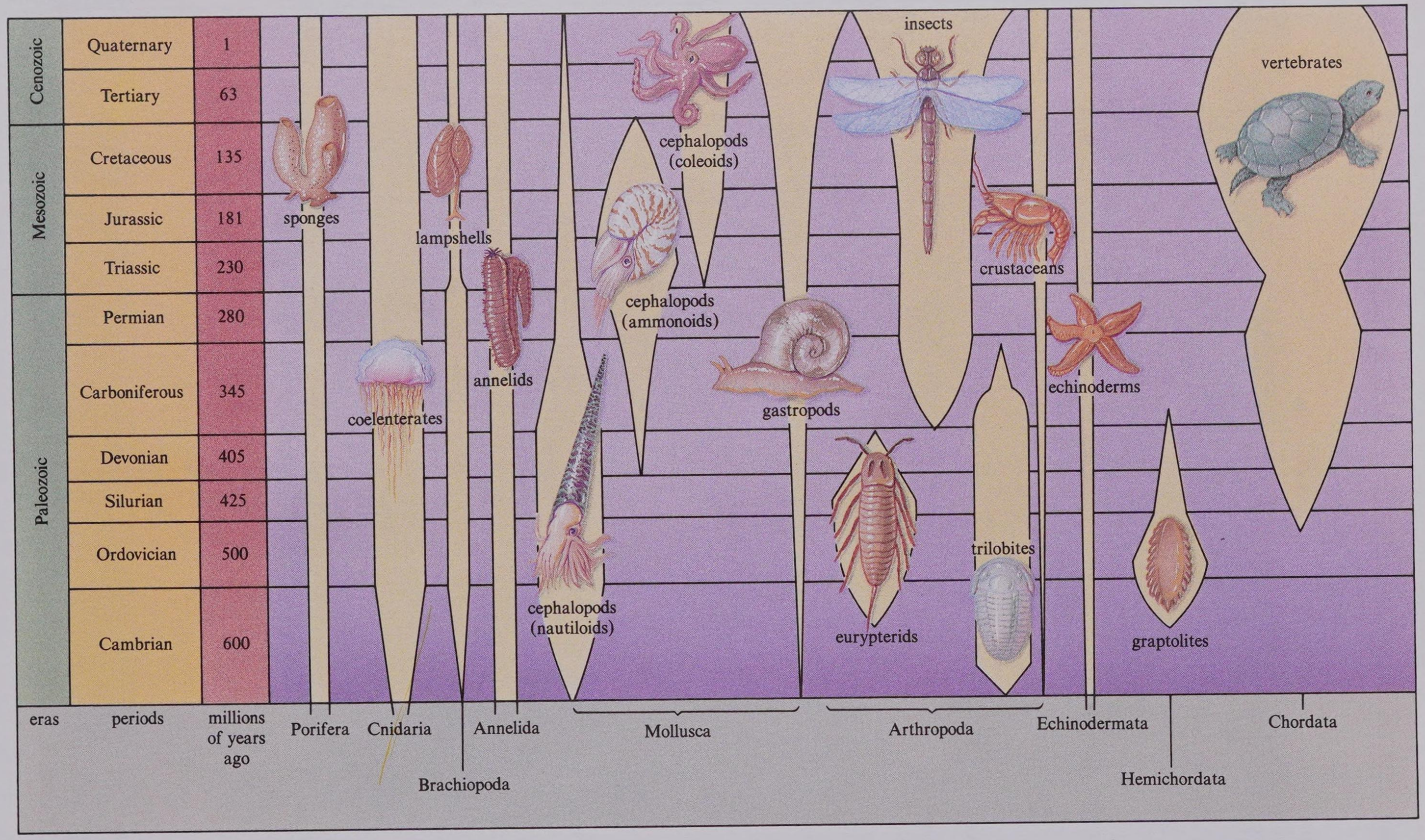
   Name the era _____ and period _____ that the first vascular plants appeared. How many million years before the present was this? _____

Figure 12.1 Geologic history of life.

Era	Period	Epoch	Years from Start of Period to Present	
Cenozoic	Quaternary	Recent	10,000	
		Pleistocene (Ice Age)	3 million	
	Tertiary		65 million	
Mesozoic "Age of Reptiles"	Cretaceous		130 million	
	Jurassic		180 million	
	Triassic		230 million	
Paleozoic	Permian		280 million	
	Carboniferous		350 million	
	Devonian		400 million	
	Silurian		435 million	
	Ordovician		500 million	
	Cambrian		600 million	
	Precambrian (Proterozoic)		1.5 billion	
			2.5 billion	
Archeozoic			4.6 billion	

	Plant Life	Animal Life
	Rise of herbaceous plants	Age of human civilization
	Extinction of many species of plants	Great mammals such as woolly mammoth and saber-toothed tiger become extinct First human social life
	Dominance of land by angiosperms	Dominance of land by mammals, birds, insects Mammalian radiation First humans
	Angiosperms prevalent, gymnosperms decline Trees resembling modern-day maples, oaks, and palms flourish	Dinosaurs reach peak, then become extinct Second great radiation of insects First primates
	Gymnosperms such as cycads and conifers still prevalent	Dinosaurs large, specialized, more abundant First mammals appear First birds appear
	Dominance of land by gymnosperms and ferns Decline of club mosses and horsetails	First dinosaurs appear Mammal-like reptiles evolve
	Gymnosperm and angiosperm(?) evolve	Expansion of reptiles Decline of amphibians Extinction of trilobites
	Age of great coal forests including club mosses, horsetails, and ferns	"Age of Amphibians" First great radiation of insects First reptiles appear
	Expansion of land plants; first forests of club mosses, horsetails, and ferns	"Age of Fishes" First land vertebrates, the amphibians First insects
	First vascular plants, modern groups of algae and fungi	First air-breathing land animals, such as land scorpion, appear Rise of fishes
	Invasion of land by plants(?)	Diverse marine invertebrates, coral and nautiloid common First vertebrates appear as fish
	Marine algae common	Diverse primitive marine invertebrates, trilobites common Animals with skeletons appear
	Multicellular acoelomate and coelomate animals evolve Eukaryotic protists and fungi evolve	
	Prokaryotes abundant	
	Anaerobic and photosynthetic bacteria evolve Formation of earth and rest of Solar System	

Geologic history of animals: fossil range and relative abundance of major phyla and selected subgroupings.



Name the era _____ and period _____ during which the dominance of the land by angiosperms (flowering plants) occurred. How many millions of years before the present was this? _____

2. Using figure 12.2 answer the following questions:

   During what period did the trilobites appear? _____

   When did they become extinct? _____

   During what period did the insects first appear? _____

   How many million years ago was this? _____ What animals were dominant during this time (fig. 12.1)? _____ What plants were prevalent? _____

S 3. Obtain a box of selected fossils for each geologic era (Cenozoic, Mesozoic, Paleozoic). The geologic time scale is shown in figures 12.1 and 12.2. Fill in table 12.1 from the identification key or the fossil labels.

Table 12.1 Fossils

Era (fill in period if available)	Type of Fossil (phylum, class, or common name)	Description (may include a sketch)
Cenozoic		
Mesozoic		

Table 12.1 *cont.*

Era (fill in period if available)	Type of Fossil (phylum, class, or common name)	Description (may include a sketch)
Paleozoic		

Embryological Evidence

Early embryonic stages of related organisms resemble each other (fig. 12.3). In many cases, embryos develop structures that do not occur in the adult. For example, among the vertebrates, all embryos develop gill pouches or slits at some point during development. Yet such structures are functional only in aquatic vertebrates, not in terrestrial ones. This has caused many biologists to conclude that, in some cases, embryos may possess structural characteristics that were functional in the organism's evolutionary history.

Ⓢ Obtain prepared slides of a chick and a pig embryo at comparable stages of development. Observe each of the embryos using a dissecting microscope. List 5 similarities between the 2 embryos.

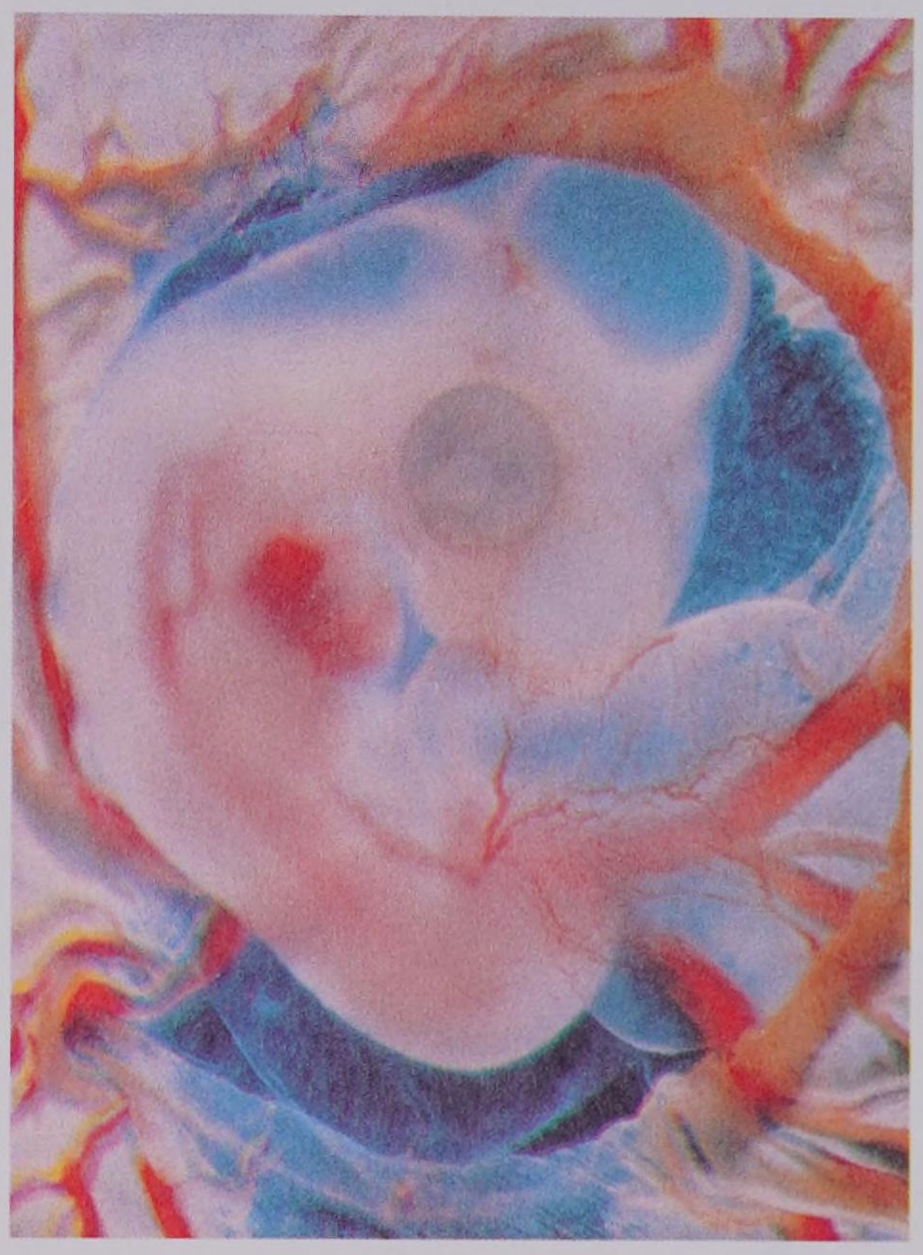
1. _____
2. _____
3. _____
4. _____
5. _____

All vertebrate embryos go through similar embryological stages. Why might that be? _____

_____

_____

Figure 12.3 Chick and pig embryo at comparable stage have many characteristics in common. ***a.*** Chick embryo. ***b.*** Pig embryo.



a.

b.

Anatomical Evidence

In the study of evolutionary relationships, organisms or parts of organisms are said to be *homologous* if they exhibit similar basic structures and embryonic origins. If these organisms or parts of organisms are similar in function only, they are said to be *analogous*. Homologous structures are derived from a common ancestor and are used to classify organisms.

Comparison of Vertebrate Limbs

The limbs of vertebrates are homologous structures. The basic pattern is the same, although there are differences, especially in the lengths of the components. The similarity of the limbs is explainable in that vertebrates are descended from a common ancestor and thus have all inherited a common pattern.

S 1. Take the central diagram in figure 12.4 as representative of the forelimb bones of the ancestral vertebrate. The basic components are the humerus (h), ulna (u), radius (r), carpals (c), metacarpals (m), and phalanges (p) in the 5 digits.

2. Study this diagram, and a series of mounted limb bones of various vertebrates, in particular the frog, lizard, bird, bat, cat, and human.

3. Carefully compare and label the corresponding forelimb bones throughout the series.

4. Take particular note of the specific modifications that have occurred in some of the bones to meet the demands of a particular way of life.

S 5. Fill in table 12.2 to indicate which bones in each specimen appear to be the most primitive (i.e., resemble the ancestral condition) and which are the most advanced (i.e., differ from the ancestral condition). Use the *letter symbols* given previously.

Figure 12.4 Vertebrate limbs compared to the ancestral condition (*center*).

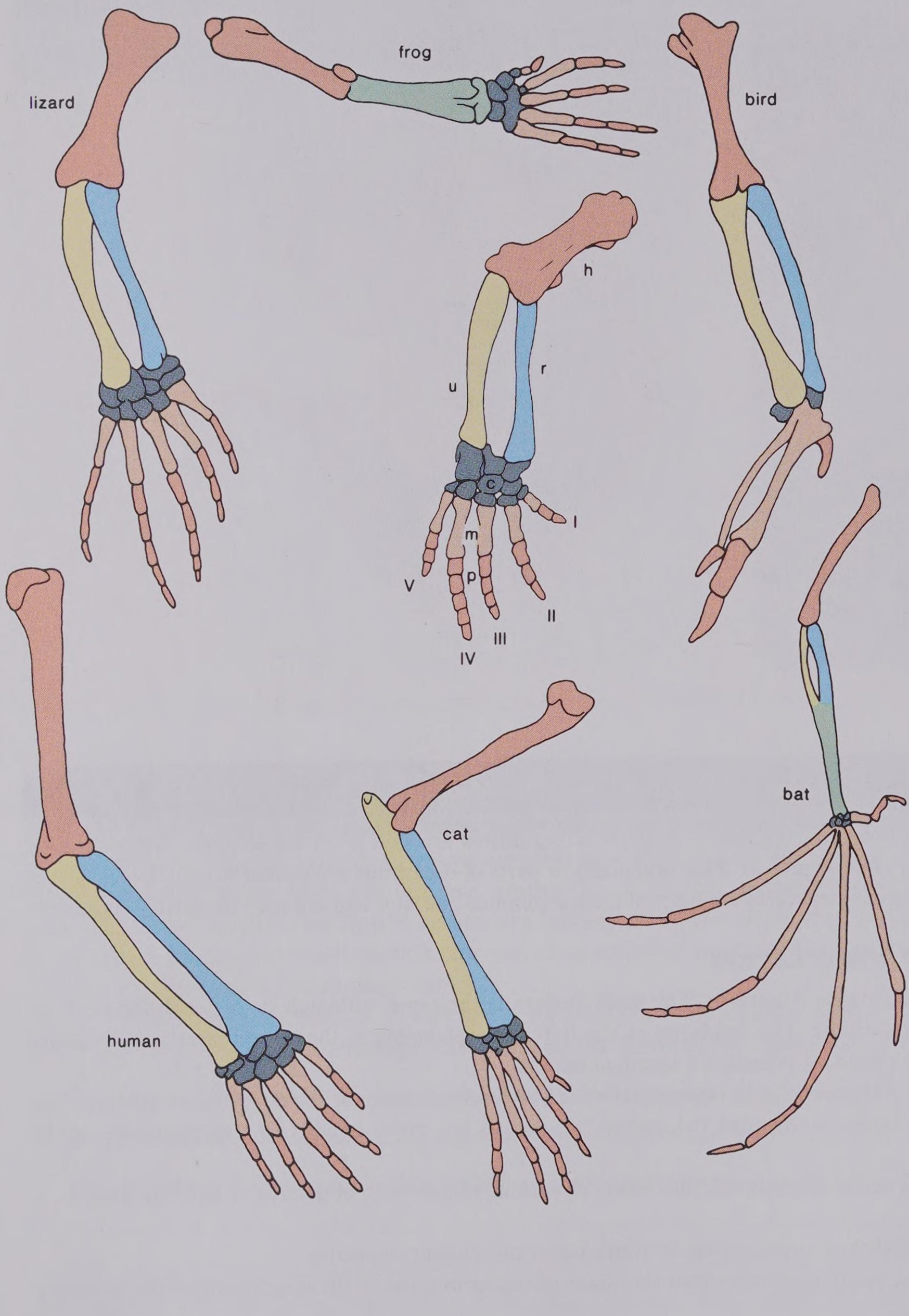


Table 12.2 Comparison of Vertebrate Limbs

Name of Animal	Bones that Resemble Common Ancestor	Bones that Differ from Common Ancestor

Relate the change in structure to a change in function if possible. Give 2 examples.

Example 1. _____

_____

Example 2. _____

_____

Comparison of Monkey and Human Skeletons (Optional)

Monkey and Human Skulls

S 1. Compare a human skull (preferably a fetal skull) with a monkey skull. The forehead region of the skull is formed by the *frontal* bone. Behind this is the pair of *parietal* bones forming the top of the head. Toward the rear of the parietals is the *occipital,* in which you will notice a large opening, the *foramen magnum.* The nerve cord passes through this opening. The rounded surfaces of the occipital, called the *occipital condyles,* articulate with the first vertebra.

Just to the side and below each parietal is a *temporal* bone, which outlines the opening to the ear and also extends forward to form part of the cheek, the *zygomatic arch.* Between the eye sockets and forming the bridge of the nose are the paired *nasals.* The bones that constitute the upper jaw are the *maxillae;* the lower jaw, or *mandible,* consists of a series of fused bones.

S 2. Examine the position and shape of the parietal bones in both the monkey and human skulls. How does the monkey skull differ from the human skull in this respect? _____

_____

S 3. Compare the shape and position of the occipital bones in the adult human and monkey skulls. _____

_____

How does the difference in the position of the foramen magnum correlate with the posture and stance of the 2 organisms?

_____

_____

_____

S 4. Compare the slope of the frontal bones of the human and monkey skulls. How are they different? _____

_____

For which skull is the supraorbital ridge, the ridge above the eye, more thickened? _____

What is the position of the mouth and chin in relation to the profile for each skull? _____

_____

What effect has the evolutionary change in the positions of these bones had on the shape of the face? _____

_____

S 5. Examine the teeth in the adult human and monkey skulls. Diet can account for many of the observed differences. Monkeys are vegetarians, and a vegetarian diet requires strong facial muscles that attach to bony projections. Humans are omnivorous but evolved as carnivorous. A meat diet does not require strong grinding teeth or well-developed facial muscles. Are the shapes and types of teeth similar? _____

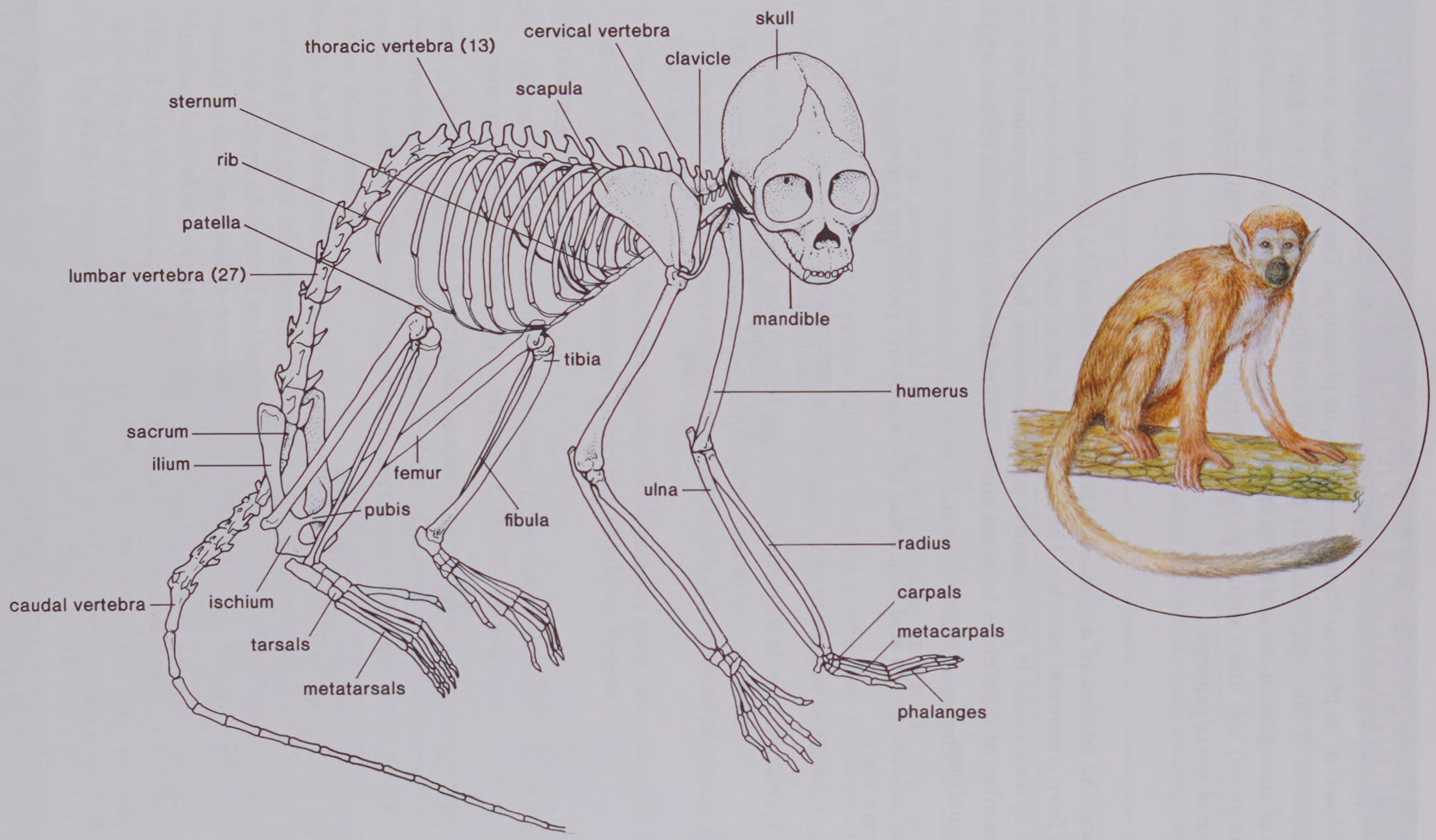
Monkey and Human Skeletons

S Compare the entire monkey (fig. 12.5) and human skeletons (fig 29.4) and complete table 12.3. Means of locomotion can account for many of the observed differences. Monkeys are arboreal and climb and live in trees; humans are terrestrial and walk erect, leaving the hands free for manual manipulation.

Table 12.3 Comparison of Human and Monkey Skeletons

Skeletal Part	Human	Monkey
Shape of spine		
Tail?		
Shape of pelvis		
Opposable first toe on foot		
Shape of clavicle		
Opposable thumb		
Length of arm relative to body		
Length of leg relative to body		
Prehensile foot (grasping)		

Figure 12.5 Squirrel monkey skeleton.



Biochemical Evidence

Every organism is recognizable by the sum of its physical features—that is, its phenotype. These physical characteristics are reflections of the chemical composition of the organisms. The chemicals are direct products of the genes. Thus in looking at the chemical components of an organism, we are really looking at its genetic components as well. Organisms that are closely related (have similar genes) will make similar proteins. Unrelated organisms will make many proteins that are unique and found only in one of the organisms.

Protein Clock

According to the *protein clock theory,* the number of amino acid changes is proportional to the length in time since 2 organisms began evolving separately from a common ancestor. The sequence of amino acids in *cytochrome c,* the carrier of electrons in the electron transport system found in mitochondria and chloroplasts, has been determined in a variety of organisms. Figure 12.6 lists the number of differences between amino acid sequences for several of these. Examine the figure and answer the following questions.

Figure 12.6 Number of differences between amino acid sequences of the protein cytochrome c obtained from a variety of organisms on the evolutionary scale.

= endothermous animals (warm-blooded animals)

= ectothermous animals (cold-blooded animals)

= fungi

	human	monkey	pig, bovine, sheep	horse	dog	rabbit	kangaroo	chicken, turkey	duck	rattlesnake	turtle	tuna fish	moth	neurospora	candida	yeast
human	0															
monkey	1	0														
pig, bovine, sheep	10	9	0													
horse	12	11	3	0												
dog	11	10	3	6	0											
rabbit	9	8	4	6	5	0										
kangaroo	10	11	6	7	7	6	0									
chicken, turkey	13	12	9	11	10	8	12	0								
duck	11	10	8	10	8	6	10	3	0							
rattlesnake	14	15	20	22	21	18	21	19	17	0						
turtle	15	14	9	11	9	9	11	8	7	22	0					
tuna fish	21	21	17	19	18	17	18	17	17	26	18	0				
moth	31	30	27	29	25	26	28	28	27	31	28	32	0			
neurospora	48	47	46	46	46	46	49	47	46	47	49	48	47	0		
candida	51	51	50	51	49	50	51	51	51	51	53	48	47	42	0	
yeast	45	45	45	46	45	45	46	46	46	47	49	47	47	41	27	0

Cytochrome c in humans is most like that of what organism? _____

From your knowledge of evolutionary history, does this seem reasonable? Explain. _____

_____

Human cytochrome c is most unlike that of what organism? _____

What does this mean in terms of evolutionary history? _____

According to the protein clock theory, have rattlesnakes and turtles been evolving separately for a longer time than have humans and monkeys? _____

How do you know? _____

_____

Antigen-Antibody Reactions

The immune system makes *antibodies* (proteins) that react with foreign proteins, termed *antigens*. The antigen-antibody reaction is specific: an antibody reacts only with its particular antigen. In the laboratory, this reaction can be observed when a precipitate appears.

The antibody-antigen reaction has been used by biochemists to determine the degree of relatedness between animals. We will simulate one of the procedures used. The procedure contains these steps:

1. Human blood proteins are injected into the bloodstream of a rabbit, and the rabbit makes antibodies against the human blood proteins. The rabbit is now said to be *sensitized*.
2. These antibodies will react strongly (determined by the amount of precipitate) against a new sample of human blood proteins.
3. These antibodies will also react against blood proteins from other animals. The more closely related these animals are to humans, the more precipitate should be formed.

Experimental Procedure

Obtain a chemplate (tray with several depressions); one bottle each of rabbit blood serum, human sensitizing serum, and human serum proteins; and one each of test sera I, II, III (known human), IV, and V.

- (S) Step 1: Put 2 drops of *synthetic rabbit serum* in each of 6 wells in the chemplate. Label the wells 1–6. Sensitize the samples of blood serum by adding 2 drops of *human sensitizing serum* to each well. Mix each well with a separate toothpick. This simulates the production of antibodies in the rabbit's bloodstream in response to the human blood proteins.
- (S) Step 2: Add 4 drops of *human blood serum* (contains human blood proteins) to the sixth well. Mix with a toothpick and note the amount of precipitate after 2 minutes.

Step 3: Add 4 drops of *serum I* to the first well, 4 drops of *serum II* to the second well, 4 drops of *serum III* (known human) to the third well, 4 drops of *serum IV* to the fourth well, 4 drops of *serum V* to the fifth well.

- (S) Mix each well with a separate toothpick.
- (T) After 2 minutes, compare each of the 5 wells to well 6. Determine which well produced the most precipitate and which produced the least precipitate.
- (S) Rank the wells from 1 (the most precipitate) to 5 (the least), and record your ranking in table 12.4.

Table 12.4 Biochemical Evidence of Evolution

Serum	Amount of Precipitate	Animal Source
I		
II		
III		human
IV		
V		

Conclusion

These test sera (supposedly) contain blood proteins from a cow, a monkey, an orangutan, a chimpanzee, and a human. These animals are listed in order of *least* related (cow) to *most* related (chimpanzee) to humans. Judging by the amount of precipitate, complete table 12.4 by indicating which serum you believe came from which animal. On what basis did you make your decision? _____

_____

Laboratory Review 12

1. What evidence suggests that organisms are related?

2. What type of biochemical reaction studied in this laboratory helps determine the degree of relatedness between 2 organisms?

3. If there are no differences in amino acid sequences in a molecule of cytochrome c found in 2 different organisms, what can you conclude about their relationship?

4. Why would you not expect a fossil buried thousands of years ago to look exactly like a modern-day organism?

5. If a characteristic is found in bacteria, fungi, pine trees, snakes, and humans, would you say that it is "old" in terms of the evolutionary time scale or "new"? Why?

6. Explain the difference between homologous and analogous structures.

References

Brooklyn College of the City University of New York. 1973. *Biology 2.* Dubuque, IA: Kendall/Hunt Publishing Company.

Hinckley, C. P., ed. 1971. *Principles, Organisms and Processes.* Dubuque, IA: Kendall/Hunt Publishing Company.

Volpe, E. P., and DeGruy, C. V. 1983. *Biology and Human Concerns,* 3d ed. Dubuque, IA: Wm. C. Brown Publishers.

Laboratory

13

Mechanisms in Evolution

Learning Objectives

Students should be able to

1. state and use the Hardy-Weinberg law;
2. explain development of resistance to antibodies.

Introduction

All the members of a species living in one locale make up a population. How can we detect the occurrence of evolution within a population? If we determine that the genetic makeup of a population has changed, then we can state that evolution has occurred. We do this by showing that the frequency of alleles in a population's *gene pool* has changed over time. A change in the frequencies indicates that evolution is occurring.

Hardy-Weinberg Law

The Hardy-Weinberg law tells us that the relative frequencies of genes do not change in large populations from one generation to the next unless there is an outside influence such as natural selection, mutation, gene flow, or nonrandom mating.

The mathematical expression of the Hardy-Weinberg law enables us to determine the frequency of all genotypes and alleles if we know the percentage of the recessive phenotype in the population:

$$p^2 + 2pq + q^2 = 1,$$

p^2 = the frequency (percentage) of the population that is *homozygous dominant,*

$2pq$ = the frequency (percentage) of the population that is *heterozygous,*

q^2 = the frequency (percentage) of the population that is *homozygous recessive.*

In this laboratory session, we will consider the members of this laboratory to be a population and will determine the percentage of individuals who are homozygous recessive, homozygous dominant, and heterozygous for ability to taste PTC and for type of earlobe.

First Generation

Determination of q^2

PTC (phenylthiocarbamide) is an antithyroid drug that prevents the thyroid gland from incorporating iodine into the thyroid hormone. The ability to taste PTC is associated with the functioning of the thyroid gland. As with many patterns of human inheritance, the nature of the relationship between "tasting" and disease is unknown. The ability to taste PTC is an autosomal trait. Tasting (T) is dominant to nontasting (t).

S 1. Taste a piece of paper impregnated with PTC. Can you taste this chemical? _____

What is your genotype? _____

What percentage of the class is unable to taste PTC? _____

2. Convert the percentage to a decimal: _____ = q^2.

Have a member of the class examine your earlobes and, with the help of figure 8.6, determine whether they are attached or unattached. Unattached (*L*) is dominant to attached (*l*).

1. Do you have attached or unattached earlobes? _____

   What is your genotype? _____

   What percentage of the class has attached earlobes? _____

2. Convert the percentage to a decimal: _____ $= q^2$.

Determination of q and p

To calculate q, it is only necessary to take the square root of q^2. Whereas q^2 = the frequency of the population that is homozygous recessive, q = the frequency of the recessive gene. Use a calculator to take the square root of q^2 (table 13.1).

Table 13.1 Determination of q

Trait	q^2	q
PTC tasting		
Earlobes		

Whereas p^2 = the frequency of the population that is homozygous dominant, p = the frequency of the dominant gene. Calculation of p is very easy once you know that $p + q = 1$ therefore $p = 1 - q$ (table 13.2).

Why is this reasonable, considering that we are dealing with one allelic pair of genes? _____

_____

Table 13.2 Determination of p

Trait	q	p
PTC tasting		
Earlobes		

Determination of 2pq and p^2

Ⓢ Fill in table 13.3 after you have done the necessary calculations using a calculator.

Table 13.3 Determination of p^2 and 2pq

Trait	p^2	2pq
PTC tasting		
Earlobes		

Summary

Ⓢ Fill in table 13.4 for one trait of your choice. The first column requires the numbers you have just calculated. The second column may be calculated by simply multiplying the population size by the frequencies.

What is the population size (number of students in the class)? _____

Table 13.4 First Generation

Genotypes	First Column	Second Column
	Genotype Frequencies	Number of Students
Homozygous recessive (q^2)		
Homozygous dominant (p^2)		
Heterozygous (2pq)		

Next Generation

According to the Hardy-Weinberg law, what will be the frequency of the genotypes in the next generation? _____

Under what assumptions? _____

Does the population have to be large? _____

Does mating have to be random? _____

Can mutation occur? _____

Can natural selection occur? _____

Can migration in and out of the population occur? _____

Are all of these stipulations ever met? _____

Future Generations

Using the frequencies just calculated (table 13.4) as a guide, your instructor will assign you a genotype for one trait only. (Students who know their own genotypes will be assigned this genotype.)

S 1. Write down your initial (P) genotype. _____

S 2. To ensure random mating, be completely uninhibited and choose anyone in the class (male or female) as your mate and approach him or her confidently. That person cannot refuse you.

S 3. Each couple will have 2 offspring. Since each member contributes one allele to each offspring, follow this procedure for each offspring. Flip a coin: if it comes up heads the allele on the left is passed on; if it comes up tails, the allele to the right is successful.

	Your contribution	**Your partner's contribution**
Offspring 1	_____	_____
Offspring 2	_____	_____

4. Now disregard each parent genotype (assume that the parents have passed away) and take the genotype of one of the 2 offspring.

S 5. Record your new F_1 genotype. _____

S 6. Proceed to a new partner and follow exactly the same procedure, recording your new genotype each time.

S 7. Continue this same procedure until you have completed 5 generations.

S 8. Now fill in table 13.5 using the F_5 information for all members of the population. Notice that this time you complete the second column first and the first column second.

Table 13.5 Sixth Generation

Genotypes	First Column	Second Column
	Genotype Frequencies	Number of Students
Homozygous recessive (q^2)		
Homozygous dominant (p^2)		
Heterozygous (2pq)		

Ⓢ 9. Compare table 13.5 with table 13.4. _____

Do your results support the Hardy-Weinberg law? Why not? _____

_____

Which of the assumptions may not have been fulfilled? _____

_____

Do the following test to help you determine whether you may be correct.

Test Procedure

Your instructor will divide the original population into 2 smaller populations. How many persons are in the new population?

_____ Each member of the population begins with the last genotype from previously.

Ⓢ Determine the genotype and allele frequencies for your new population and complete table 13.6.

Table 13.6 First Generation

Genotypes	Genotype Frequencies	Number of Students
Homozygous recessive (q^2)		
Homozygous dominant (p^2)		
Heterozygous (2pq)		

Ⓢ Now go through the 5 generations again, choosing only members of your new population.

Initial (P) genotype _____, F_1 genotype _____, F_2 genotype _____,

F_3 genotype _____, F_4 genotype _____, F_5 genotype _____.

Ⓢ After 5 generations, fill in table 13.7.

Table 13.7 Sixth Generation

Genotypes	Genotype Frequencies	Number of Students
Homozygous recessive (q^2)		
Homozygous dominant (p^2)		
Heterozygous (2pq)		

Compare table 13.7 to table 13.6. Do the results suggest that the size of the population affects genetic equilibrium? _____ The term *genetic drift* is applied to situations in which chance alone causes genetic frequencies to change. Genetic drift is usually more pronounced in small populations than in large populations. Genetic drift is a means by which evolution occurs. Evolution is sometimes defined as a _____ in gene pool frequencies.

Antibiotic Selection of Bacteria

If bacteria are grown in the presence of small amounts of antibiotics such as penicillin, most die but some survive. Penicillin acts by interfering with cell wall synthesis. The bacteria that survive have a different set of enzymes for cell wall synthesis than those that die; therefore, it can be reasoned that surviving bacteria also have genes that are different from genes in bacteria that died. One or more of the genes for cell wall synthesis has undergone a mutation *prior to exposure to the antibiotic,* and this mutation makes the bacteria resistant to the antibiotic.

In the experiment we will perform, each bacterium that survives will be allowed to produce a colony of cells. Thus, the antibiotic environment will select these cells to reproduce and will bring about a change in gene pool frequencies.

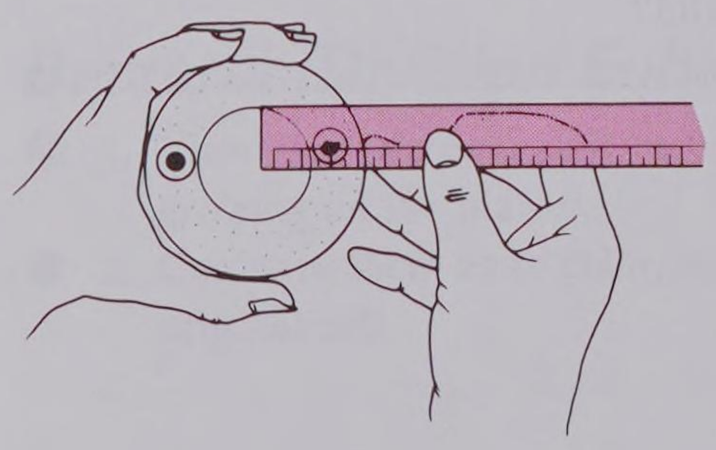
Experimental Procedure

1. Obtain 2 culture plates that have already been inoculated with *E. coli* bacteria.
2. Watch as your instructor places on each plate a blank sterile disc to serve as a control and 2 discs that are impregnated with antibiotic.
3. Place a capital letter on the bottom of each plate beneath each disc; use C for control and use the first letter of each antibiotic being tested.
4. Write your name and lab section on the top of one plate and tape it closed.
5. Before closing the second plate, expose it for 10 seconds to an ultraviolet lamp enclosed in a box to cause mutations. (*Caution:* Direct UV exposure will injure the eyes.)
6. Place the plates in an incubator at 37° C for 24 hours.
7. Examine the plates after incubation. If you cannot examine the plates at the end of that time, they will be placed in a refrigerator until you can observe them. Measure the clear zones (indicates bacterial death) between the edges of the discs and the edges of growth (fig. 13.1) and record your data in table 13.8.
8. Compare your data to data of other members of the laboratory.

Table 13.8 Response to Antibiotic

	Control Disc	Antibiotic	Antibiotic
Plate (not exposed)			
Plate (exposed to UV)			

Figure 13.1 Measurement of the clear zone of bacterial death from disc edge to edge of growth indicates the effectiveness of the antibiotic.



9. Answer the following questions:

What caused the growth of colonies around the antibiotic discs? _____

_____

If bacteria from these colonies alone were used to repeat the experiment, how would your results differ? _____

_____

Would a comparison of the first generation with this next generation show a change in genotype frequencies?

_____

Is this an example of natural selection and adaptation? Explain. _____

_____

Laboratory Review 13

1. What is the evidence that evolution is occurring in any given population?

2. If $p = 0.8$, what is the value of q?

3. If $q = 0.5$, what percentage of the population is homozygous for the recessive allele?

4. Does mutation causing bacterial resistance occur before or after exposure to antibiotic?

5. If $q = 0.4$, what is the frequency of the heterozygous genotype?

6. What conditions are necessary in order for the Hardy-Weinberg law to be valid?

Reference

Trelease, et al. 1980. *Biology 101*. Dubuque, IA: Kendall/Hunt Publishing Company.

Laboratory

14

Diversity I: Bacteria, Protista, and Fungi

Learning Objectives

Students should be able to

1. identify visually all organisms studied;
2. classify each organism as to kingdom, common category (i.e., bacteria, protozoa, etc.), and division/phylum;
3. state the significant features of all organisms studied.

Introduction

To study and compare organisms, we classify them into groups according to their accepted evolutionary relationships. The smallest group is the *species;* within this group, organisms share the same structure and can usually interbreed and produce fertile offspring. Closely related species are placed in the same *genus*. The genus and species names constitute the *scientific name* of an organism. For example, *Homo sapiens* is the scientific name for human beings. (Notice that the genus is capitalized and the species is not capitalized. Both are italicized.) Closely related genera are placed in the same family and so forth up to the largest category of all—*kingdom*. The major categories of classification are as follows:

Kingdom Phylum/Division Class Order Family Genus Species

There are several different classification systems in current use. The classification system utilized in this manual is given in the appendix C.

Kingdom Monera

Bacteria, including cyanobacteria (formerly called blue-green algae), are the simplest of the organisms to be studied. Their cells are *prokaryotic* and lack the organelles found in the *eukaryotic* cells studied in Laboratory 3. For example, while the cell does contain DNA, there is no defined nucleus; and while some of the organisms have thylakoids (membranous bags or discs), there is no chloroplast. Reproduction is primarily asexual by *fission* (cell division without a spindle apparatus). Most bacteria are *saprophytic,* meaning that they send out digestive enzymes into the environment and thereafter take up the resulting nutrient molecules. Some bacteria are *parasitic* and cause diseases such as strep throat or gangrene. Other bacteria are *photosynthetic* or *chemosynthetic* and thus are able to make organic molecules utilizing inorganic molecules. Cyanobacteria are always photosynthetic. They contain chlorophyll, but the green color is often masked by other pigments. In fact, some cyanobacteria are red, brown, or even black.

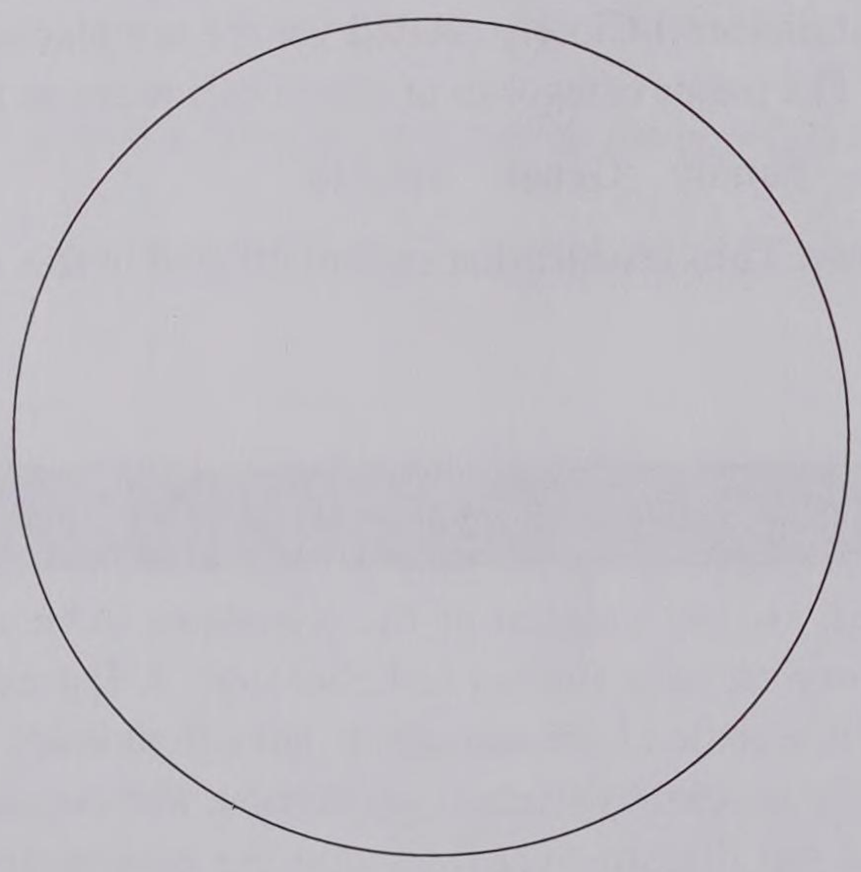
Bacteria (Division Eubacteria)

S 1. View agar plates that have been inoculated with bacteria and then incubated. Notice the "colonies" of bacteria growing on the plates.

S 2. Compare them as to color, surface, and margin. Each colony contains cells that are all descended from one original cell.

S 3. *Sketch* the colonies in the space provided.

S 4. Label the *species name, color, texture,* and *margin* for each available species.
S 5. If available, obtain a sterile agar plate and *inoculate* the plate with your thumbprint; or use a swab and inoculate the plate with material from around the teeth or from inside the nose, a garbage can, the locker room floor, or the cafeteria, etc.
S 6. Put your name on the plate and place it where directed by your instructor. It will be *incubated* and returned to
I you next laboratory period. These exposed plates may have been previously prepared. If so, continue on to the next procedure.
S 7. Obtain your incubated agar plate that was exposed during the last laboratory period.
S 8. Sketch the colonies that have grown in the space provided. Label it according to each colony's *color, surface,* and *margin.*



S 9. View the microscope slides that are on display. Notice the magnification required to view bacteria and note it here:

_____

Bacteria may be classified simply on the basis of their differences in shape (fig. 14.1). Three commonly recognized shapes are *bacillus* (rod-shaped), *coccus* (round or spherical), and *spirillum* (spiral-shaped).

Do any of the slides on display show bacterial cells with endospores? _____ What is the name of this bacterium? _____

These are not spores in the ordinary sense in which the term is used, meaning reproductive cells, but are ordinary bacterial cells in which the contents have become condensed; therefore, they serve as resistant resting cells.

Figure 14.1 Scanning electron micrographs of bacteria. ***a.*** Spherical-shaped bacteria. ***b.*** Rod-shaped bacteria. ***c.*** Spiral-shaped bacteria with flagella used for locomotion.

a.

b.

c.

The electron microscope shows the makeup of a bacterial cell. The electron micrograph in figure 14.2 indicates that the cell is bounded by a cell wall (the composition of this wall is different from that of the plant cell wall). The plasma membrane lies very close to the cell wall; in the cytosol it is possible to make out ribosomes and the nucleoid, where the bacterial chromosome is found.

Cyanobacteria (Division Eubacteria)

Cyanobacteria were formerly called blue-green algae because their general growth habit and compound light microscope appearance is similar to green algae. Electron microscopic study of them revealed that they are structurally similar to other bacteria, particularly other photosynthetic bacteria.

Gleocapsa

Prepare a wet mount of a culture, if available, or examine a prepared slide of *Gleocapsa* using high power (45×) or oil immersion (if available). The cells adhere together because each is surrounded by a sticky gelatinous sheath (fig. 14.3). What is the estimated size of a single cell? _____ (Refer to Laboratory 1 or Laboratory 3 to recall how to make this computation.)

Figure 14.2 Bacterial cell. ***a.*** Electron micrograph. ***b.*** Drawing. (***b:*** From Brown and Wishard, *Biology Laboratory Textbook*. Copyright 1974 by Kendall/Hunt Publishing Company.)

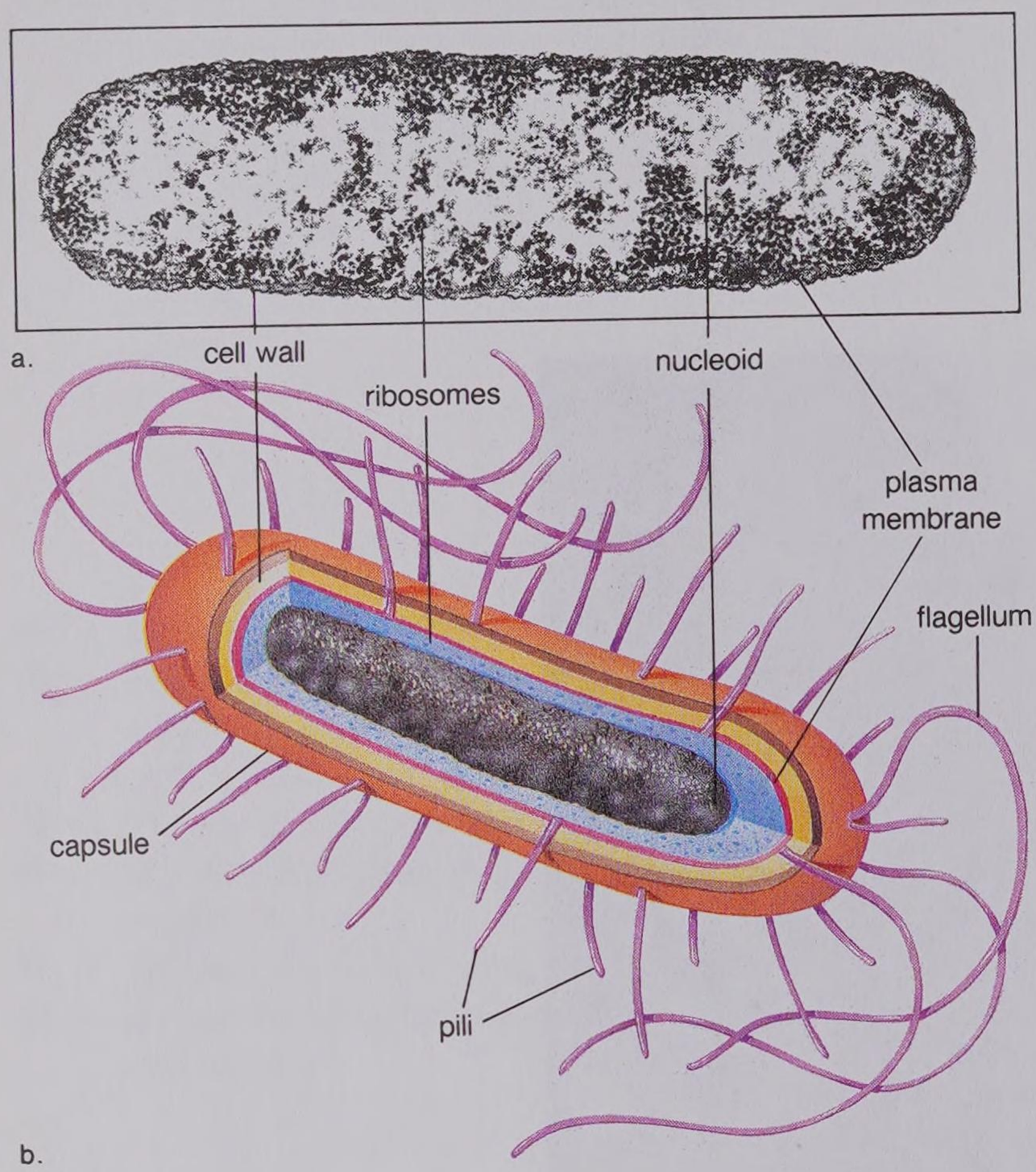


Figure 14.3 *Gleocapsa.* ***a.*** Drawing. ***b.*** Photomicrograph.

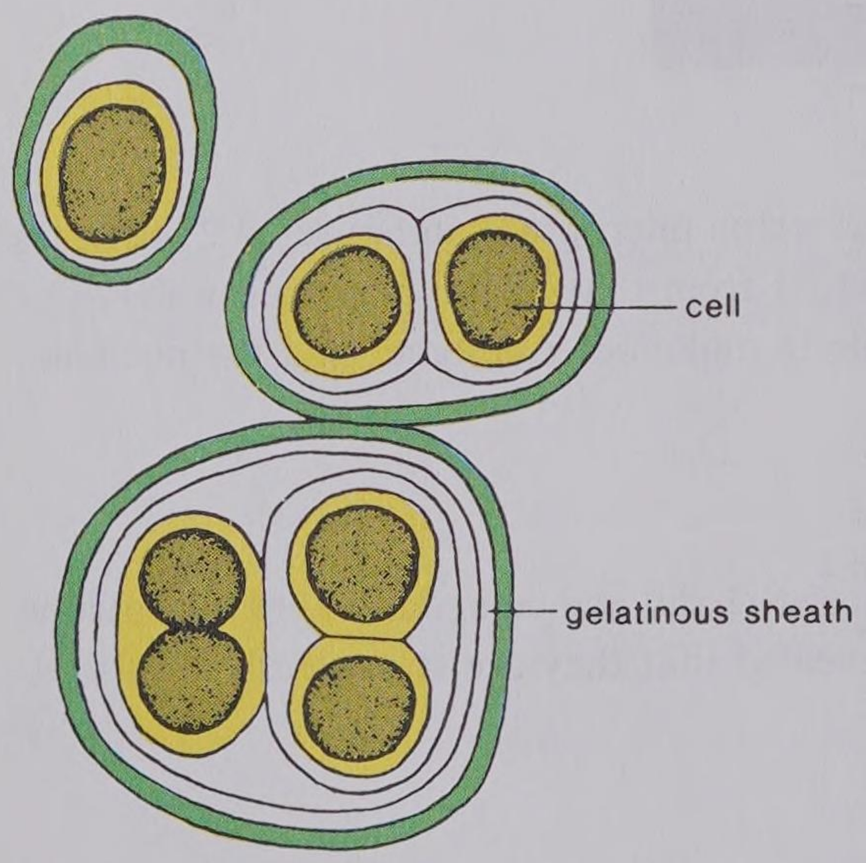
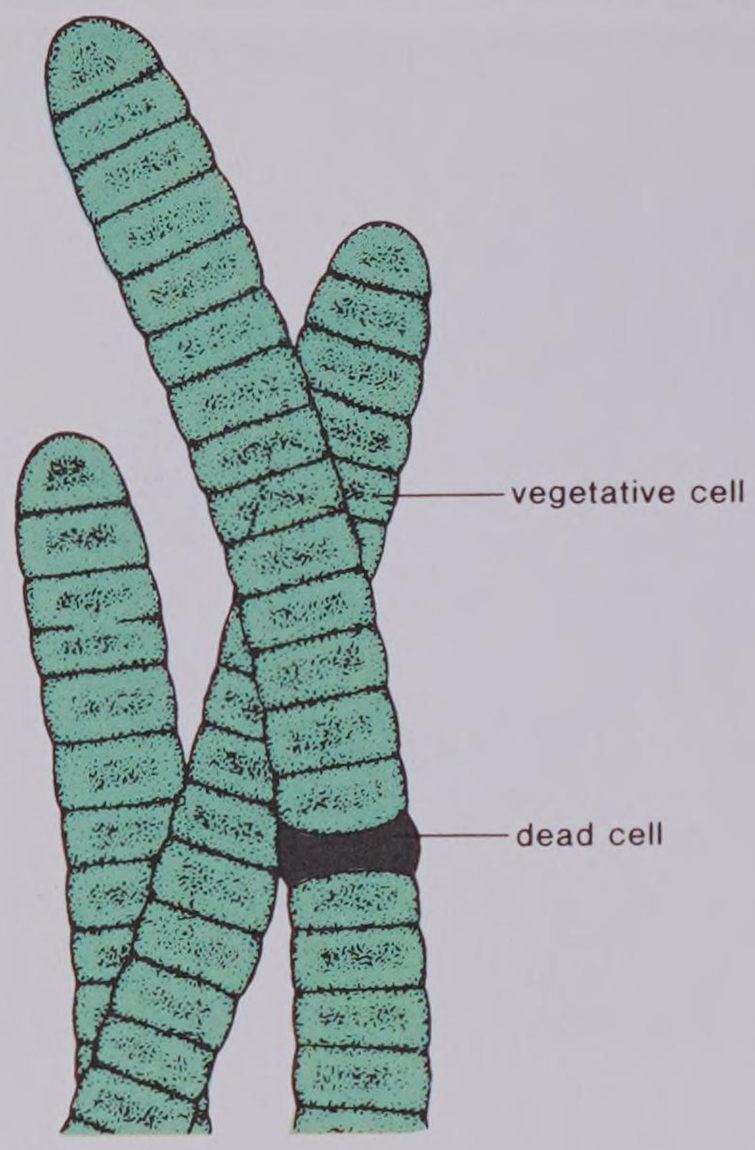
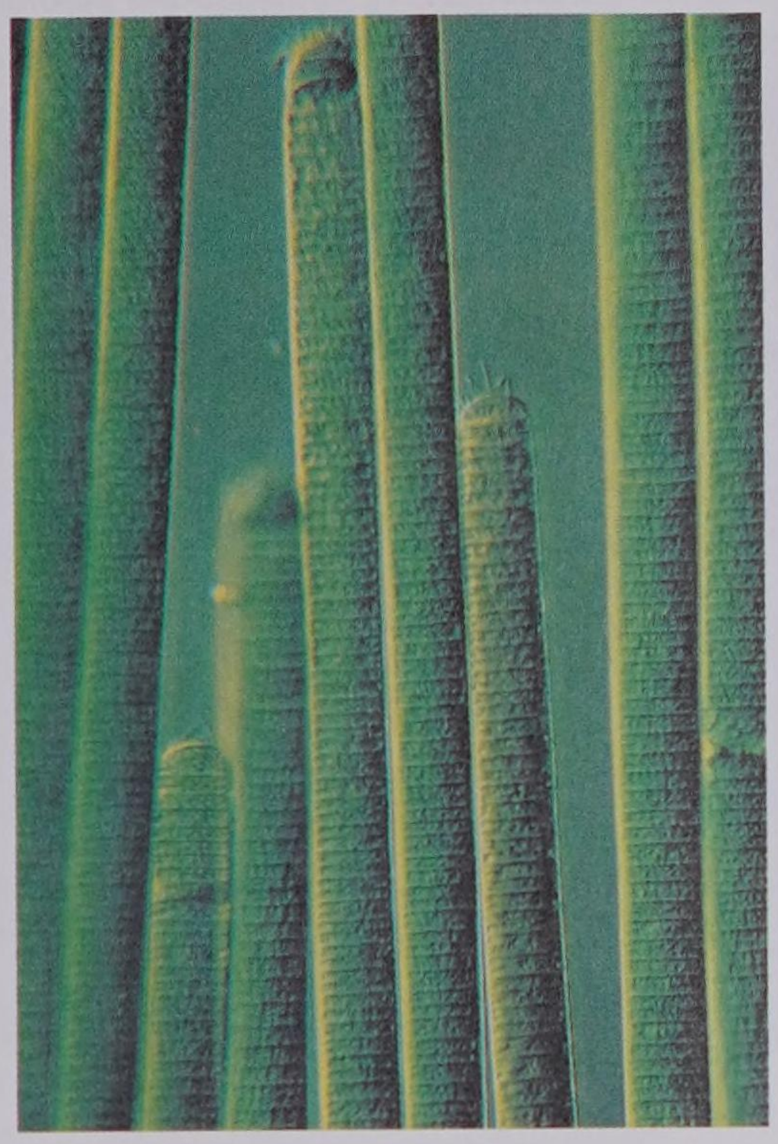


Figure 14.4 *Oscillatoria*. **a.** Drawing. **b.** Photomicrograph.



a.



b.

Oscillatoria

Ⓢ Prepare a wet mount of a culture, if available, or examine a prepared slide of *Oscillatoria* using high power (45×) or oil immersion (if available). This is a filamentous cyanobacterium with individual cells that resemble a pile of pennies (fig. 14.4).

Oscillatoria takes its name from the characteristic oscillations that you may be able to make out if your sample is alive. Do you have a living culture? _____ If yes, is this the motion visible? _____

Anabaena

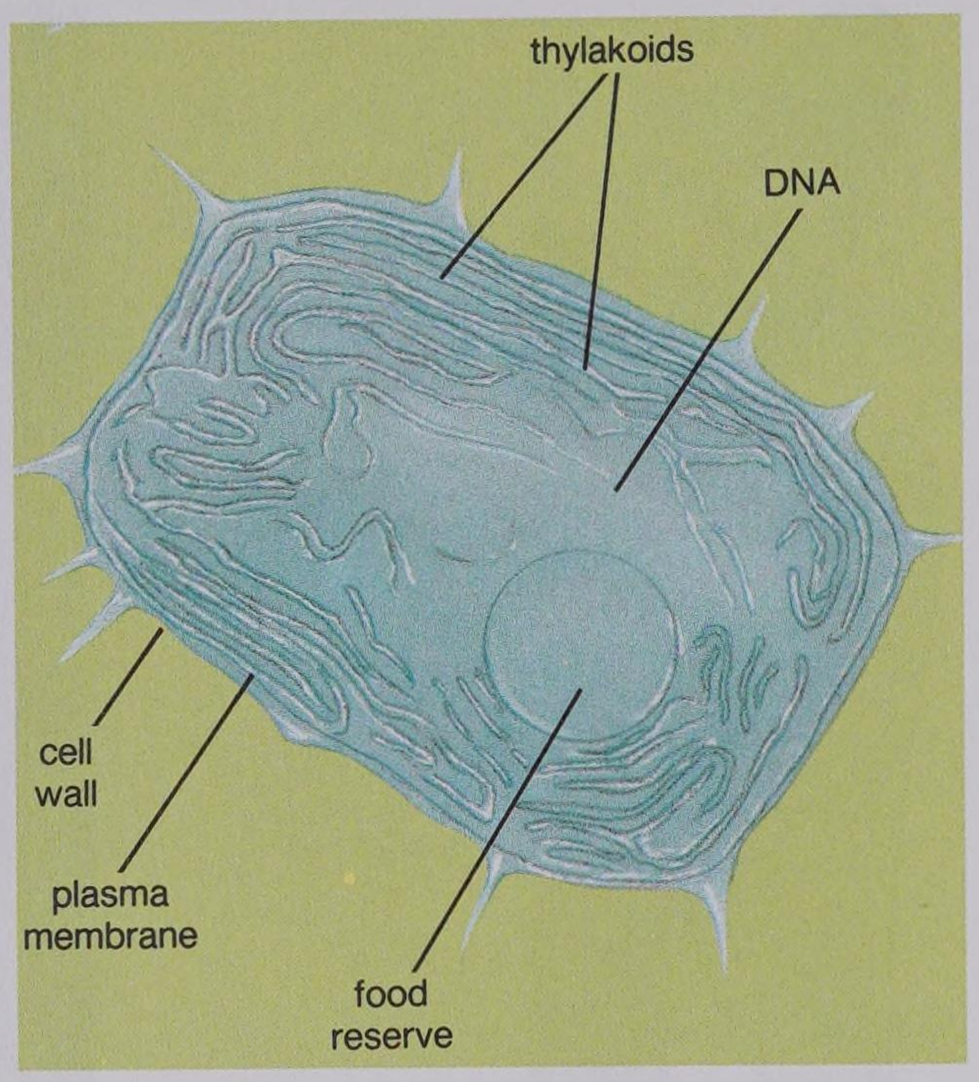
Ⓢ 1. Prepare a wet mount of a culture, if available, or examine a prepared slide of *Anabaena* using high power (45×) or oil immersion (if available). This is also filamentous cyanobacterium, with its individual cells barrel-shaped.

2. Note the thin nature of this strand. If you have a living culture, what is its color? _____ (Prepared slides are artificially stained and may not resemble the color of the living cyanobacterium.)

The cells of *Anabaena* have been studied with the electron microscope. In figure 14.5, you can make out the same structural details as before plus thylakoid membranes where photosynthesis occurs. Cyanobacteria carry on photosynthesis but don't have chloroplasts. Explain. _____

Figure 14.5 *Anabaena.* **a.** Scanning electron micrograph. **b.** Interpretive illustration.

a.



b.

Kingdom Protista

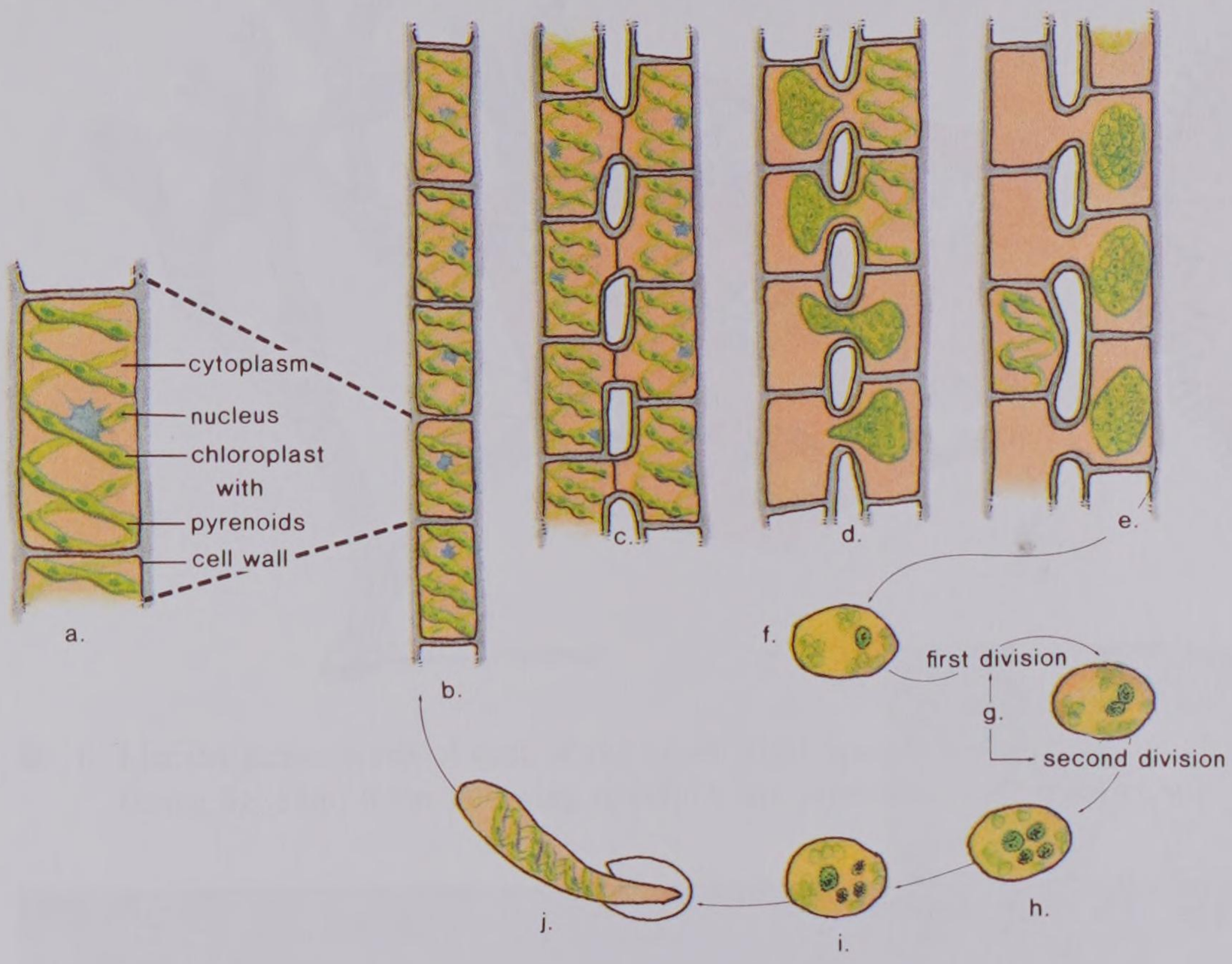
Protozoans

Free-living protozoans are typically single-celled organisms that engulf whole food, forming *food vacuoles*. Other vacuoles are also typical, such as *contractile vacuoles* that rid the cell of excess water, especially in freshwater species. Protozoans can be classified according to means of locomotion; some locomote using *pseudopodia,* some by *cilia,* and some by *flagella*. Others, such as one that causes a common form of malaria, do *not locomote* at all. They live intracellularly as parasites.

You may have already had the opportunity to observe *Amoeba* (p. 31) (phylum Sarcodina) and *Paramecium* (p. 13) (phylum Ciliophora); however, your instructor may wish for you to observe these organisms again.

S 1. If a *film loop* or *video* is available, observe it and note the various forms of protozoans.
S 2. Prepare *wet mounts* or examine prepared slides as directed by your instructor.
S 3. *Sketch* the available protozoa, and write its *name* beneath the sketch and any comments about its *behavior.*

Figure 14.6 *Spirogyra.* ***a.*** Enlarged cell. ***b.*** Filament. ***c.*** Conjugation begins. ***d.*** Cell contents move through conjugation tubes. ***e.*** Zygotes have formed. ***f, g, h, i.*** Meiosis is occurring. ***j.*** Germination to give a new filament. (***b–j:*** Copyright 1975 by Kendall/Hunt Publishing Company. Reprinted with permission.)



Algae

Algae is a term used for aquatic organisms that photosynthesize like terrestrial plants yet are structurally less complex and lack conductive tissue (xylem and phloem) found in most terrestrial plants. They are commonly named for the type of pigment they contain; for example, there are green, golden-brown, brown, and red algae. All algae contain green chlorophyll, but they may also contain other pigments that mask the color of chlorophyll. Algae are grouped according to their pigmentation, as well as according to their biochemical differences, such as the chemistry of the cell wall and the chemical compound used to store excess food.

Green Algae (Phylum Chlorophyta)

It is sometimes suggested that the green algae are ancestral to the first plants because both of these groups possess chlorophylls a and b, both store reserve food as starch, and both have cell walls that contain cellulose.

Ⓢ 1. If available, view a *film loop* or *video* showing the many forms of green algae.

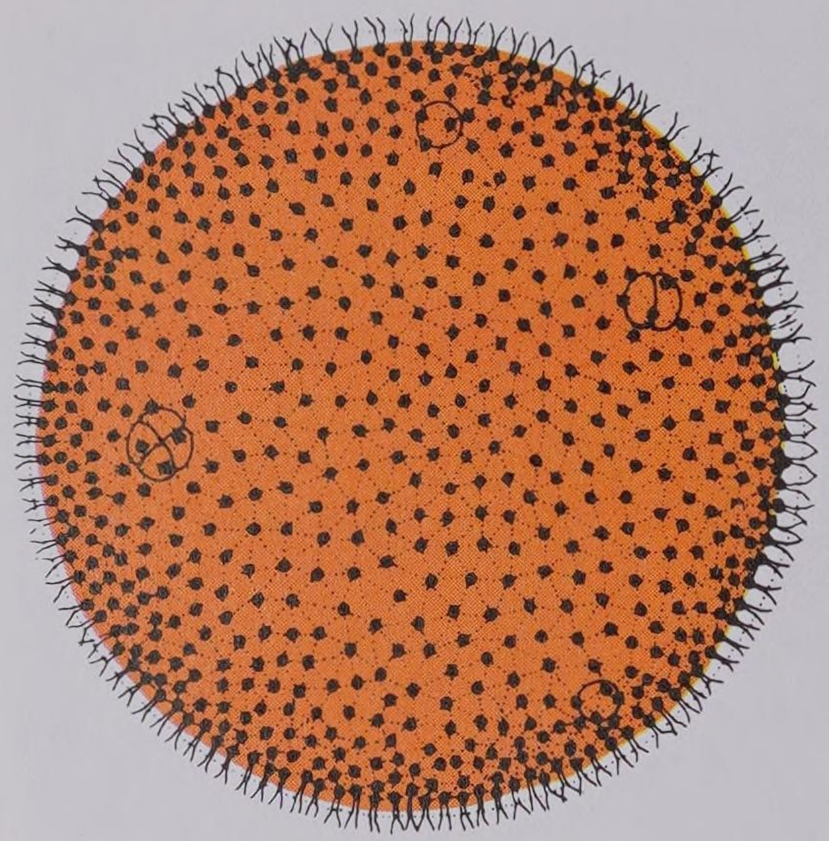
Ⓢ 2. Notice that green algae can be *single* cells, *filaments, colonies,* or multicellular *sheets.* We will examine a filamentous and a colonial form. *Spirogyra* lives in fresh water and is often seen as a bright green, slimly, filamentous cluster on or near the surface of ponds and lakes.

Ⓢ 3. Make a wet mount of live *Spirogyra* and/or observe a prepared slide. The most prominent feature of the cells is the spiral, ribbonlike chloroplast (fig. 14.6). How do you think *Spirogyra* got its name? _____

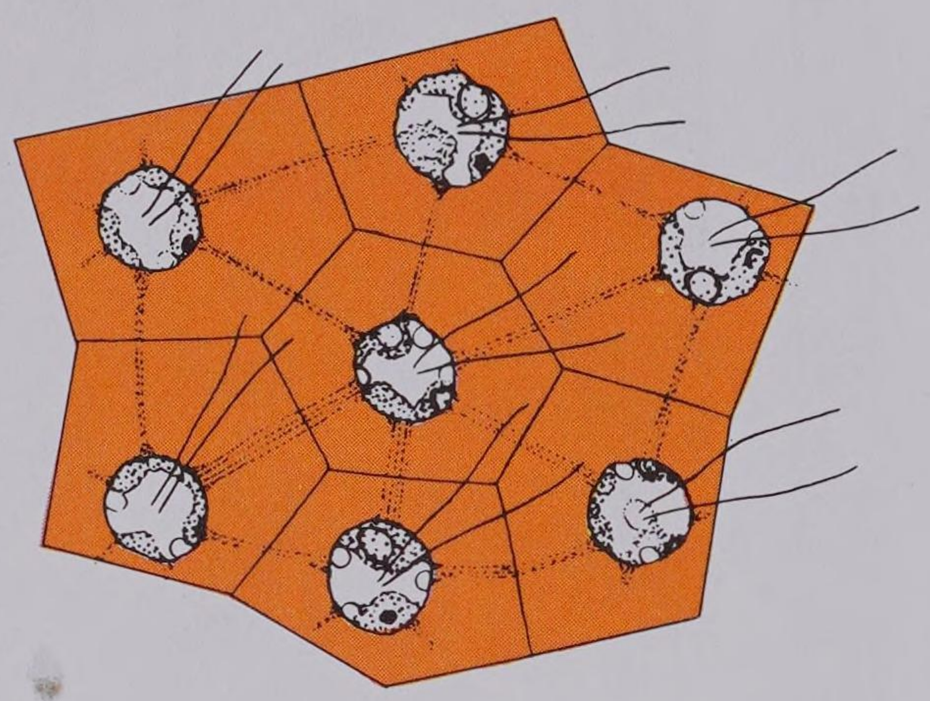
The chloroplast contains a number of circular bodies, the *pyrenoids,* which are centers of starch production. The nucleus is in the center of the cell, anchored by cytoplasmic strands.

Ⓢ Your slide may show *conjugation,* a sexual means of reproduction illustrated in figure 14.6*d*. If it does not, *obtain* a slide that does show this process. Conjugation tubes form between 2 adjacent filaments, and the contents of one set of cells enters the other set. As the nuclei fuse, a zygote is formed. The zygote overwinters, and in the spring it germinates after meiosis takes place.

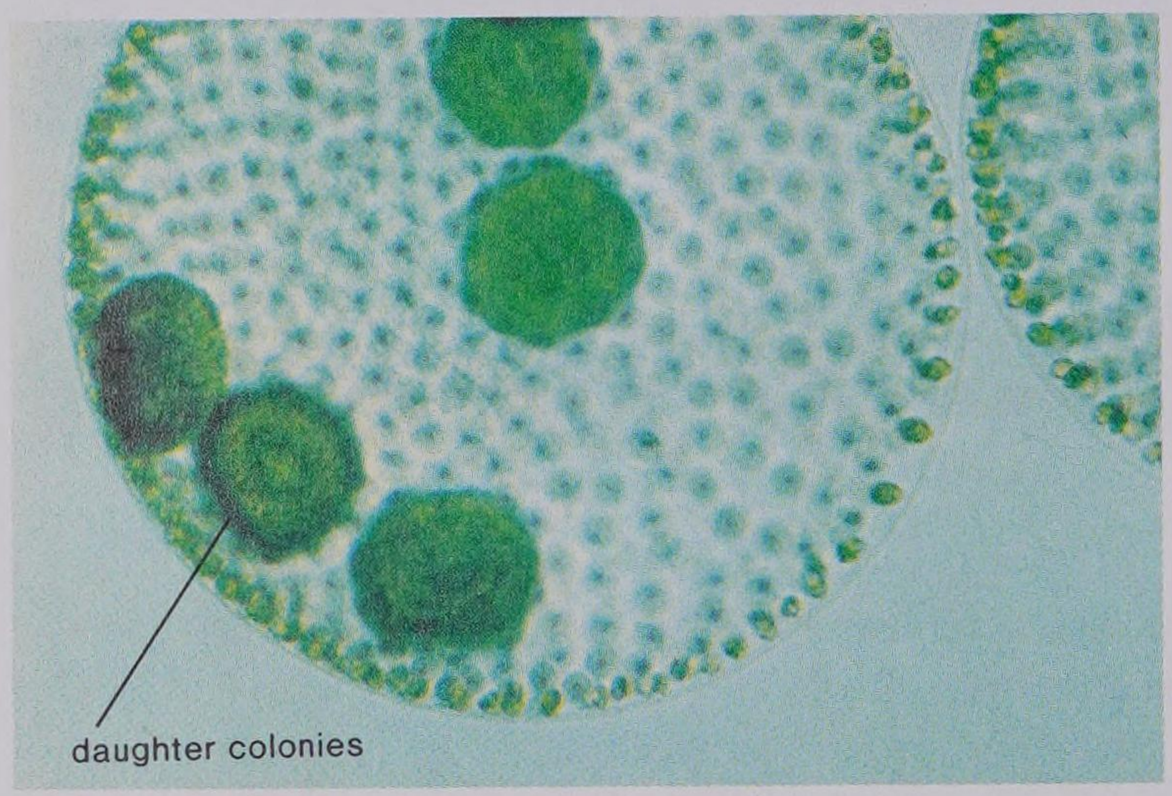
Figure 14.7 *Volvox*. **a.** Adult colony. **b.** Individual cells. **c.** Daughter colonies.



a.



b.



c.

Ⓢ 4. *Volvox* is a green algal colony (fig. 14.7). Using a depression slide, make a wet mount of live *Volvox* or study the prepared slide. Volvox is motile (capable of locomotion) because the thousands of cells that make up the colony have flagella. These cells are connected by delicate cytoplasmic extensions (fig. 14.7*b*).

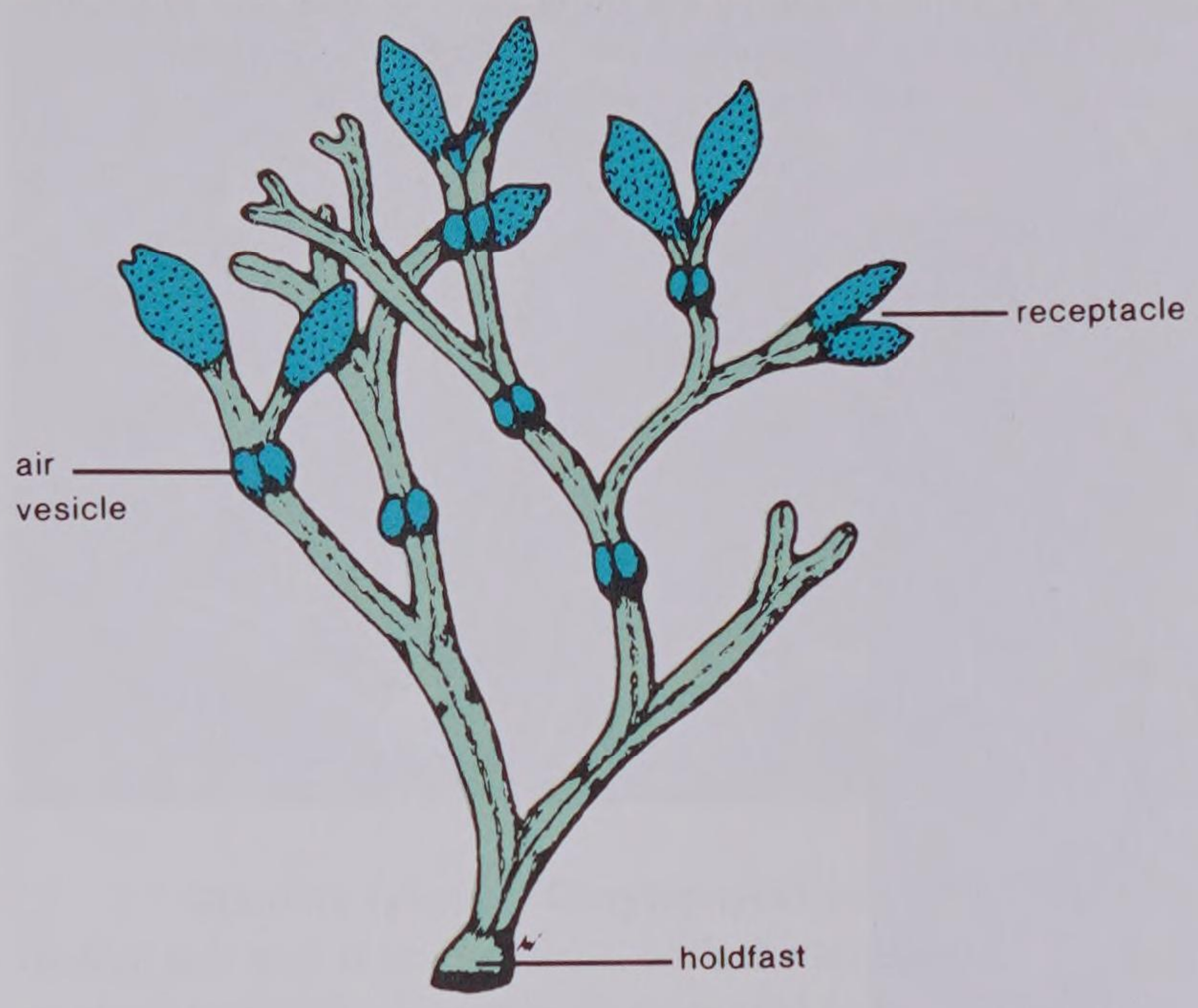
Volvox is capable of both asexual and sexual reproduction. Certain cells of the adult colony can divide to produce *daughter colonies* (fig. 14.7*c*) that reside for a time within the parental colony. A daughter colony leaves the parental colony by releasing an enzyme that dissolves away a portion of the matrix of the parental colony, allowing it to escape. During sexual reproduction, some colonies of *Volvox* have cells that produce sperm, and others have cells that produce eggs.

Brown Algae (Phylum Phaeophyta)

Brown algae, commonly called *seaweed,* along with the multicellular green and red algae, contain a brown pigment that masks the green color of chlorophyll. These algae are large in size and have specialized parts.

Ⓢ *Fucus* is called rockweed because it is seen attached to rocks at the seashore when the tide is out (fig. 14.8). If available, view a *preserved specimen.* Note the body plan, called dichotomously branched, since the thallus (body) repeatedly divides into 2 branches. Note also the holdfast by which the alga anchors itself to the rock, the air vesicles or bladders that help hold the thallus erect in the water, and the receptacles or swollen tibs. The receptacles are covered by small raised areas, each of which has a hole in the center. The areas are cavities in which the sex organs are located, with the gametes escaping to the outside through holes. *Fucus* is unique among algae in that it is 2N and always reproduces sexually. The large brown algae, such as *Sargassum* and *Laminaria,* are called *kelps*.

Figure 14.8 *Fucus* thallus.



❶ 1. List the genus names of each of the brown algae specimens available and identify and check off in table 14.1 (using fig. 14.8) if the following structures are present on your specimens: *holdfast, air vesicle,* and *receptacle.*

Table 14.1 Brown Algae

Specimen No.	Name (genus)	Holdfast	Air Vesicle	Receptacle
1				
2				
3				
4				

Red Algae (Phylum Rhodophyta)

Like most brown algae, the red algae are multicellular, but they occur chiefly in warmer sea water, growing both in shallow waters and as deep as light penetrates. Some forms of red algae are filamentous, but more often they are complexly branched with a feathery, flat, and expanded or ribbonlike appearance (fig. 14.9*b*). Coralline algae are red algae that have cell walls impregnated with calcium carbonate ($CaCO_3$).

❶ List the *genus* names of each of the red algae specimens available, and *sketch* and *label* the color in the following space:

Figure 14.9 ***a.*** Representative red alga (*Sebdenia polydacria*). Red algae are smaller and more delicate than brown algae. Both are economically important because colloidal substances extracted from the outer layer of their cell walls are used in foods to produce a smooth consistency and help retain moisture. One colloid, agar, is used in culture media for bacteria and fungi. ***b.*** *Corallina.* A red algae that contains calcium carbonate. When dry and bleached, it is often mistaken for the true coral that are animals (phylum Cnideria).

a.

b.

Unicellular Algae

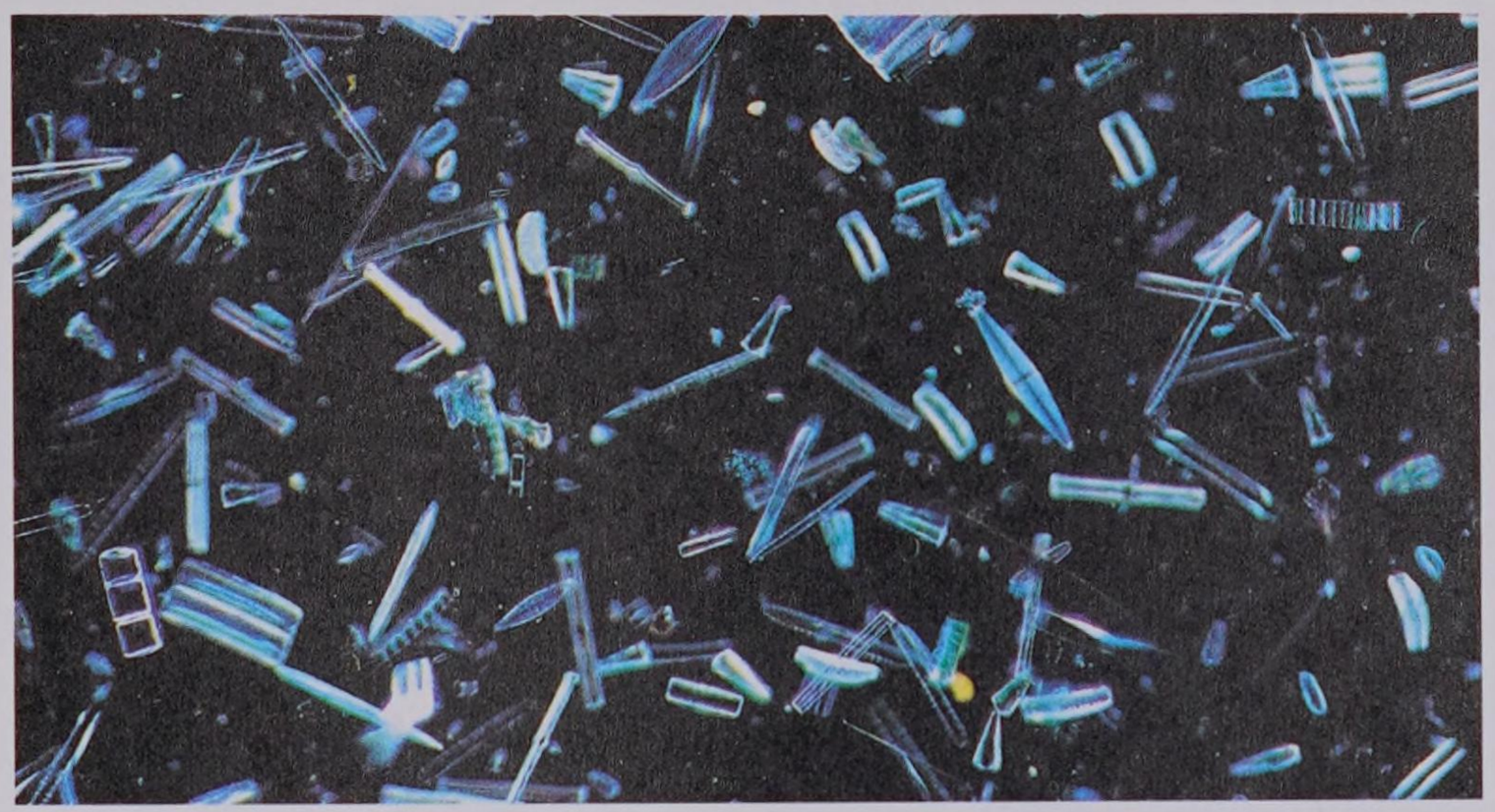
There are several groups of exclusively unicellular algae in the kingdom Protista. They also contain chloroplasts and carry out photosynthesis in a manner similar to plants.

Euglenoids (phylum Euglenophyta) are freshwater organisms having both animal-like and plant-like characteristics. Their animal-like characteristics include motility and a flexible body wall. They move by means of flagella, and because they are flexible, they can take different shapes.

S 1. Make a wet mount of live *Euglena,* or view a prepared slide.

S 2. If you have a living sample, examine it carefully under high power (45×) and see if you can determine if *Euglena* pulls or pushes itself through the water. Which does it do? _____

Figure 14.10 Diatoms.



Diatoms (phylum Chrysophyta) possess a yellow-brown pigment in addition to chlorophyll (fig. 14.10). The diatom cell wall is in 2 sections, with the larger one fitting over the smaller as a lid fits over a box. Since the cell wall is impregnated with silica, diatoms are said to "live in glass houses." The glass cell walls of diatoms do not decompose, so they accumulate in thick layers that are subsequently mined as diatomaceous earth and used in filters and as a natural insecticide. Diatoms, being photosynthetic and extremely abundant, are very important sources of food for the small heterotrophs (organisms that must acquire food from external sources) in both marine and fresh water.

S 3. Make a wet mount of live diatoms or view a prepared slide.
S 4. *Sketch* 2 of these diatoms in the space following:

Pond Water (Optional)

S 1. Obtain a sample of *pond water* and prepare a wet mount. Be sure to select some of the sediment on the bottom and a few strands of filamentous algae.

S 2. Sketch _____ (number as directed by the instructor) the organisms in the space provided. (And on additional white paper if so instructed.)

S 3. Obtain an illustrated manual of pond water organisms, and with the help of your instructor *identify* the organisms you find. Write the *genus* and *phylum* or common category name (i.e., Chrysophyta/diatoms) by each sketch.

S 4. Attempt to obtain *diversity* in the organisms that you sketch by selecting them from the different groups studied.

Slime Molds (Phylum Myxomycota)

There are 2 types of slime molds: *cellular* and *acellular*. They both have an amoeboid stage that normally lives on rotting logs or dead agricultural crops. In cellular slime molds, the "slime" is composed of individual amoeboid cells. The young amoeboid cells of the cellular slime mold *Dictyostelium discoideum* grow and divide by binary fission as long as food and water supplies are adequate. If the food supply is depleted, they collect into a sluglike multicellular mass that begins to push upward, forming a migrating slug. The slug is sensitive to environmental conditions such as temperature and light intensity. If there is an increase in illumination, the slug begins to form a stalk. The stalk is a fruiting body that produces spores. Wind, rain, or an animal disperse the spores into a new environment where the cycle can begin over again.

- S 1. Obtain a plate of *Dictyostelium* (cellular) or *Physarum* (acellular) growing on agar.
- S 2. Examine the plate carefully under the dissecting microscope.
- S 3. Describe what you see. _____

_____

Slime molds are said to have funguslike characteristics, but they do not have a filamentous (mycelium) growth habit like most true fungi, yet they live on dead organic matter and produce windblown spores during one of the life cycle stages.

Summary of Protista

Protozoans, algae, and slime molds are all in the kingdom Protista.

Which of these is most like animals? _____ Why? _____

_____

Which of these is most like plants? _____ Why? _____

_____

Which of these is most like fungi? _____ Why? _____

_____

Kingdom Fungi

Fungi are nongreen and therefore do not carry on photosynthesis. The body of a fungus is called a *mycelium* and is composed of many *hyphae,* or individual strands. Sometimes the nuclei within a hypha are separated by walls, and sometimes they are not.

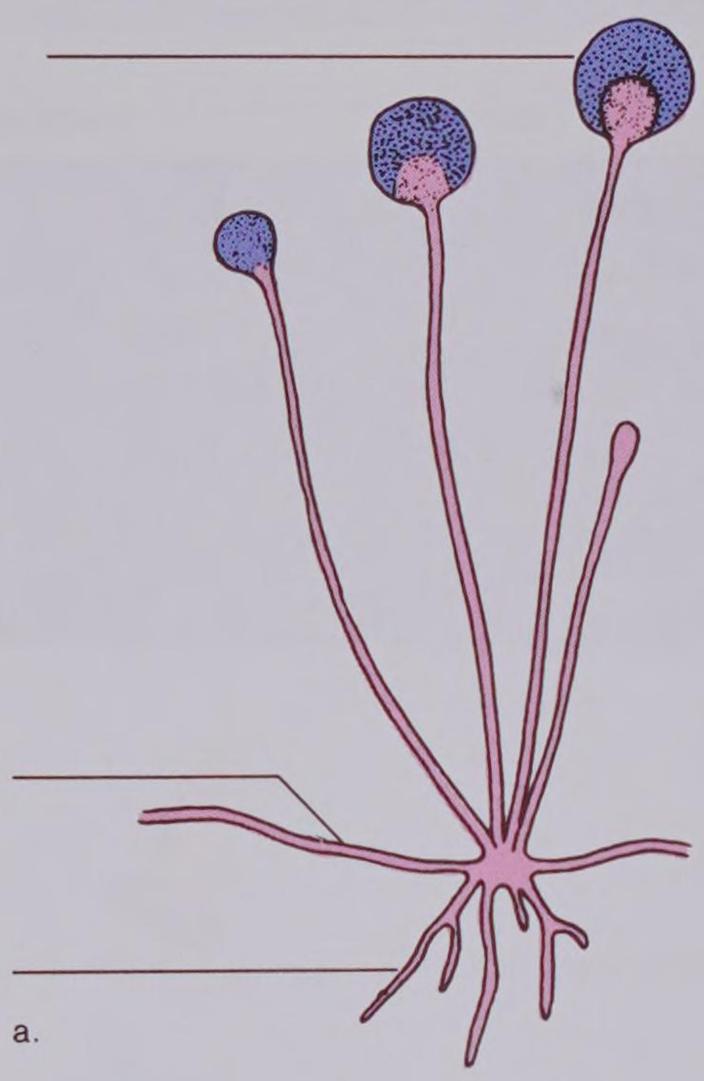
Spores, small haploid bodies with a protective covering, are produced by fungi when they reproduce sexually or asexually. Spore formation takes place within specialized hyphae. Following sexual union, a collection of specialized hyphae, called a *fruiting body,* is found in some groups.

Fungi are saprophytic in the same manner as bacteria. These 2 are often referred to as *organisms of decay.* They serve an important function by breaking down dead organic matter.

Bread Mold (Division Zygomycota)

- S 1. Observe black bread mold *Rhizopus* growth on agar medium.
- S 2. Note the cottony appearance of the mycelium and the black dots that are the spores.
- S 3. View the mold under the dissecting microscope and note the hyphal filaments making up the mycelium. The mold has 3 types of hyphae (fig. 14.11*a*): the *rhizoid* hyphae penetrate the medium; *horizontal* hyphae (stolon) run parallel to the medium; and *aerial* hyphae produce the sporangium, where spores form.
- S 4. View a prepared slide of *Rhizopus* (fig. 14.11*c*). If the slide illustrates asexual reproduction, note only the hyphae, sporangium, and spores. If the slide illustrates sexual reproduction, note in addition the *zygospores* (the zygote enclosed by a thick roughened wall).
- S 5. Label the *rhizoid, stolon,* and *sporangium* in figure 14.11*a*.

Figure 14.11 *Rhizopus.* ***a.*** Types of hyphae. ***b.*** Asexual reproduction. ***c.*** Sexual reproduction.

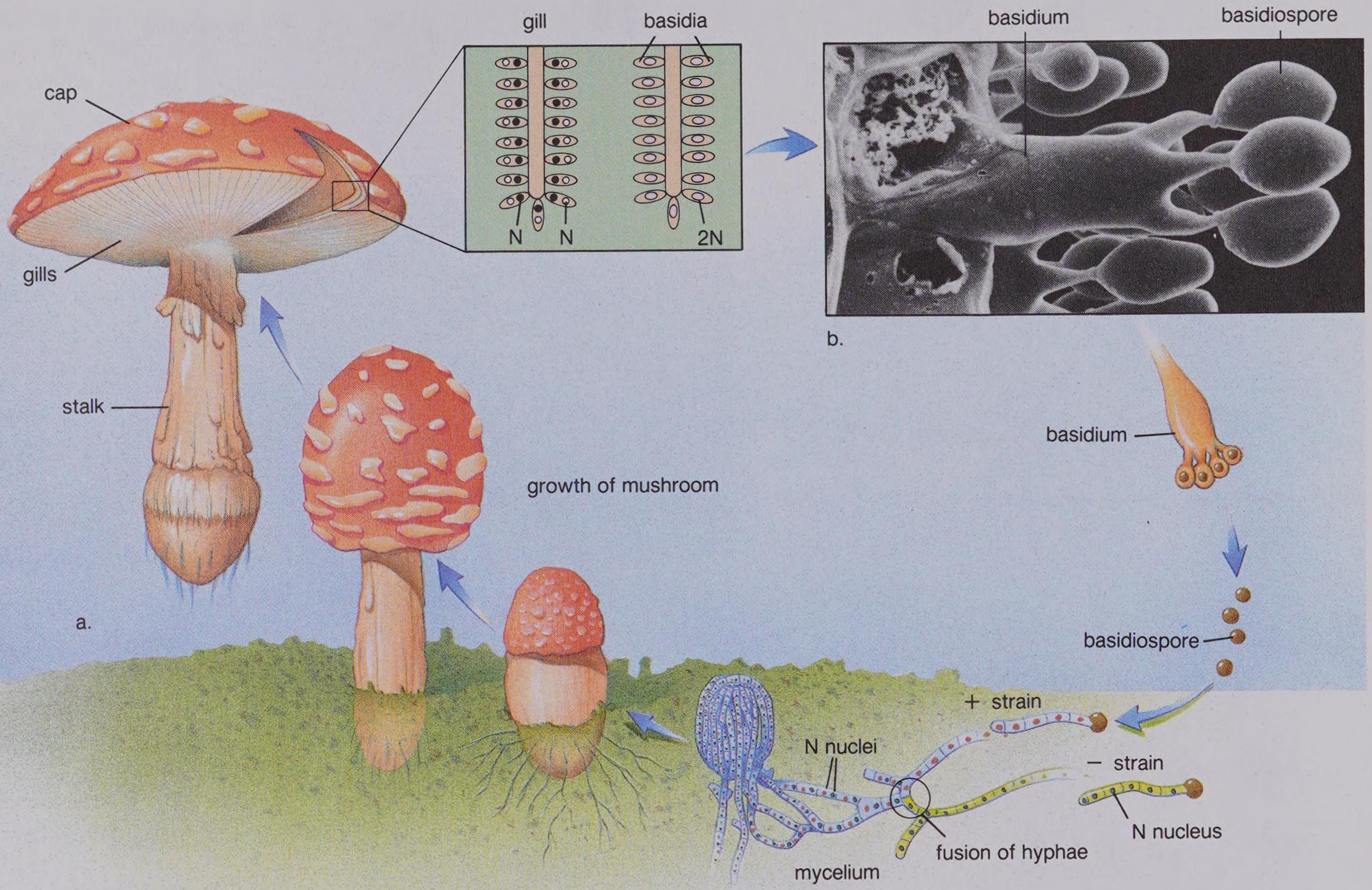


Sac Fungi (Ascomycota)

Most of the sac fungi produce asexual spores called *conidia*. The blue-green molds, *Penicillium,* grow on many different organic substances such as fruit, bread, fabrics, leather, and wood. *Penicillium* molds are used in the production of antibiotics as well as in the cheese industry to produce the characteristic taste of Camembert and Roquefort cheeses.

S 1. Obtain a petri dish of a *Penicillium* culture. Use the dropper from a bottle of methylene blue to make a streak of stain 2.5 cm long.
S 2. Obtain a 4 cm piece of clear tape. *Do not* touch the center of the tape with your fingers.
S 3. Place the center of the tape over the *Penicillium* culture so that a sample of the mold sticks to the tape.
S 4. Place the tape directly on the slide containing the methylene blue. Use your microscope to examine the slide.
S 5. Sketch what you see in the following space.

Figure 14.12 Life cycle of mushroom. A mushroom is a fruiting body whose gills are lined with basidia where meiosis produces basidiospores that germinate into hyphae. When the hyphae of 2 different strains fuse, a mushroom begins to grow.



Club Fungi (Division Basidiomycota)

The club fungi include forms familiar to the layperson such as the *mushrooms, bracket fungi,* and *puffballs.* These structures that rise above the substratum are actually fruiting bodies containing many club-shaped structures called *basidia* (fig. 14.12), where spores are produced.

S 1. Study a preserved mushroom or, if available, a live mushroom. What species of mushroom are you examining?

_____ What color are the gills? _____

S 2. Note that the underside of the mushroom cap contains gills. The basidia develop on the gill margins. At the tips of the basidia are the spores. The mushroom does have a well-developed mycelium, which lies beneath the surface.

S 3. Set an open mushroom cap on a white paper for a short time. Are spores visible? _____

What color are they? _____

Laboratory Review 14

_____ 1. What type of nutrition is found among fungi?

_____ 2. What are the 3 major groups of the Protista?

_____

_____

_____ 3. What are the different methods of movement found among protozoans?

_____

_____

4. With so many bread mold spores in the air, why isn't everything exposed covered with bread mold?

5. What is the major difference between prokaryotic and eukaryotic cells?

6. How are the 3 types of Protista seemingly related to the 3 multicellular kingdoms?

7. How can the cyanobacteria live in an environment that lacks organic nutrients?

Laboratory

15

Diversity II: Nonseed Plants

Learning Objectives

Students should be able to

1. identify the specific structural characteristics of each plant group;
2. identify the gametophyte and sporophyte generation of all plants studied;
3. describe the structure, chromosome number, and function of each generation for each plant;
4. evaluate each plant's adaptation to a dry land environment.

Introduction

Plants are believed to be directly descended from green algae because the latter organisms possess both chlorophyll *a* and *b*, have cell walls of cellulose, and store glucose as starch as the plants do. Plants differ from algae in that they always protect the zygote from drying out. Unlike algae, most plants are adapted to living on land. This feature is our primary focus as we survey the plant kingdom in this laboratory and the following laboratory. Adaptation to a dry land environment includes the characteristics listed in table 15.1.

Table 15.1 Adaptation to a Dry Land Environment

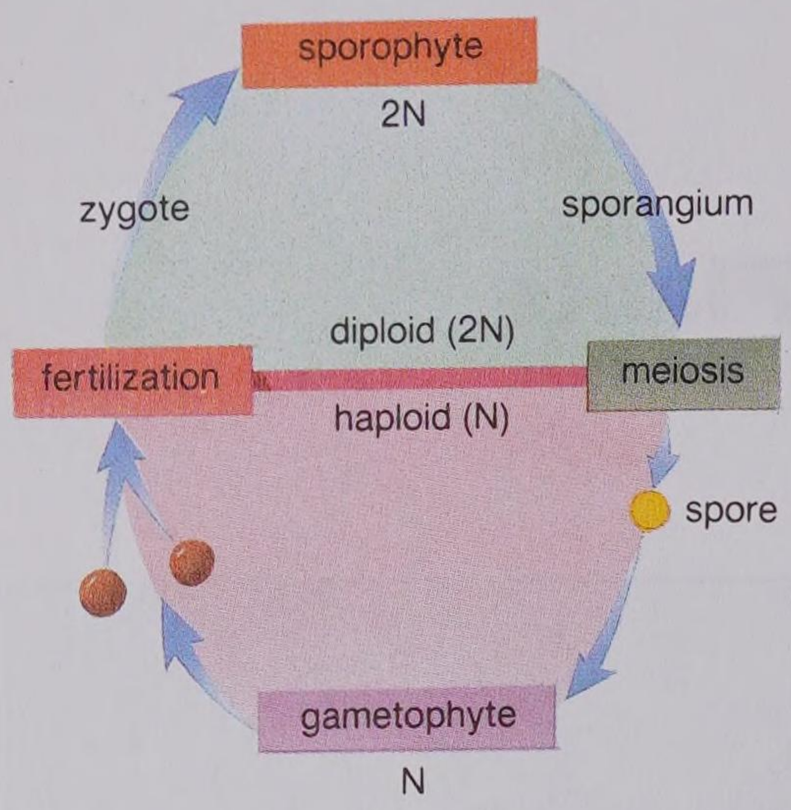
Organisms that are adapted to a dry land environment

1. obtain enough water when fluid no longer bathes the entire surface of the body;
2. transport water and nutrients throughout the body of the organism;
3. prevent excessive loss of water by a protective covering;
4. must have structural support provided by the organism;
5. carry out reproduction without dependence on external water;
6. maintain a constant internal environment.

Plants are organisms that

1. contain chlorophyll *a* and *b* and carotenoids and also store reserved food as starch and have cellulose cell walls;
2. lack the power of motion or locomotion by means of contracting fibers;
3. are multicellular and have cells specialized to form tissues and organs;
4. have sex organs with an outer layer of nonreproductive cells to prevent desiccation of developing gametes;
5. protect the developing embryo from drying out by providing it with water and nutrients within the female reproductive structure;
6. have a life cycle that is described as alternation of generations (fig. 15.1).

Figure 15.1 Alternation of generations.



Alternation of Generations

In the alternation-of-generations life cycle there are 2 mature stages known as the *sporophyte generation* and the *gametophyte generation.* The sporophyte generation produces spores, by the process of meiosis, in structures called sporangia. Spores develop into a gametophyte generation. The gametophyte generation produces gametes that later fuse to form a zygote. A zygote develops into the sporophyte generation, completing the cycle.

In plants, the sporophyte generation is the only generation that has vascular tissue, tissue that transports water and nutrients and also provides support. The sporophyte is also the 2N, or diploid, generation.

Which generation (sporophyte or gametophyte) is best adapted to a dry environment? _____

Which generation would be dominant in a widely dispersed land plant? _____

Bryophytes (Division Bryophyta)

The bryophytes (mosses and liverworts) were the first plants to live on land and are not fully developed to live in a dry environment. The gametophyte generation is dominant in these plants, which lack vascular tissue entirely. The gametophyte produces eggs within archegonia and swimming sperm in antheridia. The bryophytes are dependent on external moisture to ensue fertilization because the sperm must swim to the egg. The zygote developing within the archegonia is protected from drying out, however. In the bryophytes, the sporophyte generation is nonvascular and dependent on the gametophyte generation. Many of the spores that disperse the species, and give rise to the gametophyte generation, are windblown, though others forcefully expell their spores as they dry out.

Life Cycle of Moss, Mnium

Gametophyte Generation

S 1. Study the diagram of the life cycle of the moss (fig. 15.2) and find the gametophyte generation.

S 2. Examine a living gametophyte generation or a plastomount of this generation.

S 3. Describe its appearance. _____

_____

Considering the fact that this is the generation we refer to as "moss," what generation is dominant in these plants? _____

S 4. Study the prepared slide of the top of the female shoot, called the female head, and the top of the male shoot, called the male head.

S 5. Find the *archegonia* (fig. 15.3) at the top of the female shoot and locate an egg in at least one of these.

Figure 15.2 Life cycle of the moss.

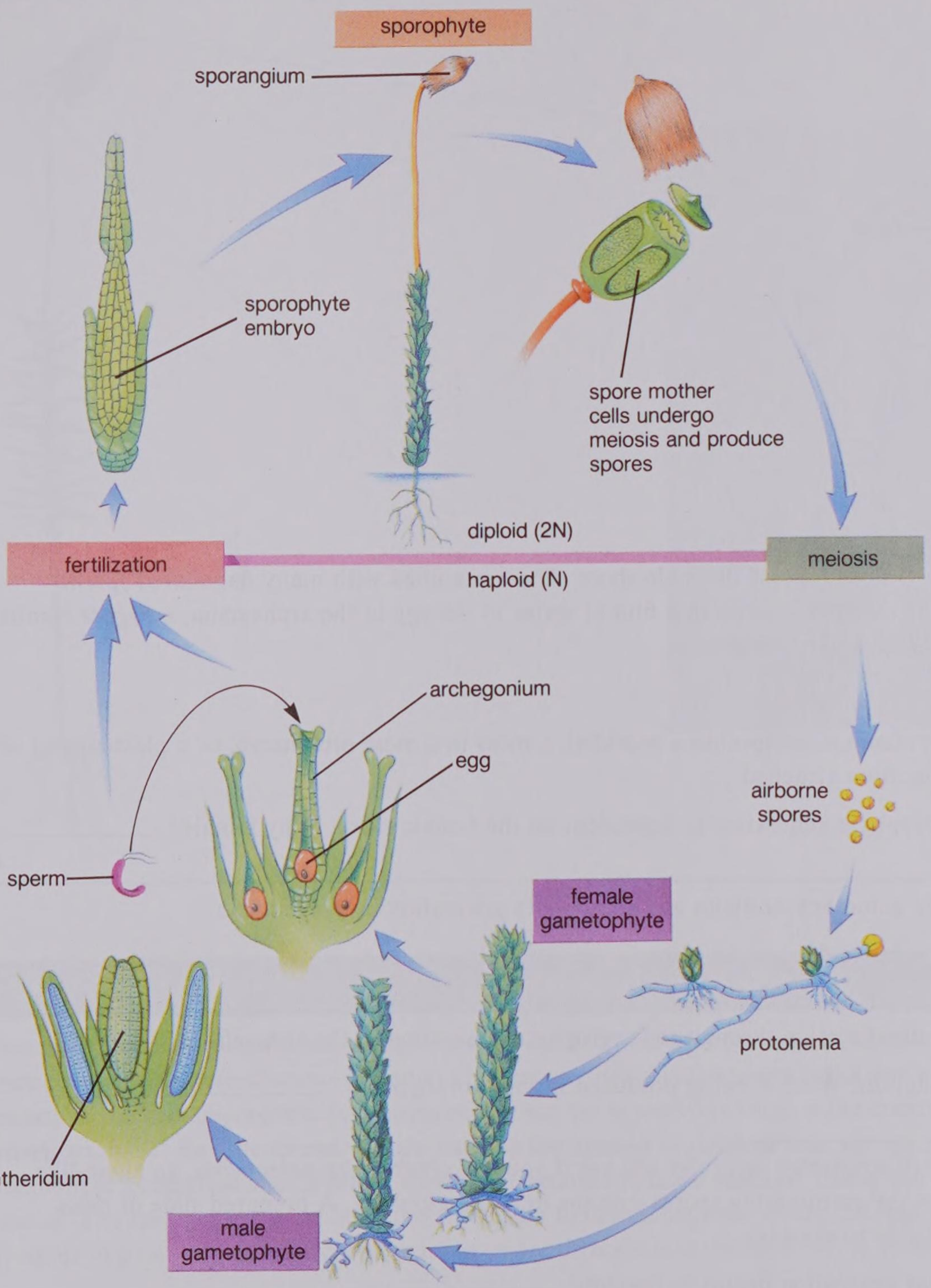


Figure 15.3 Moss archegonia.

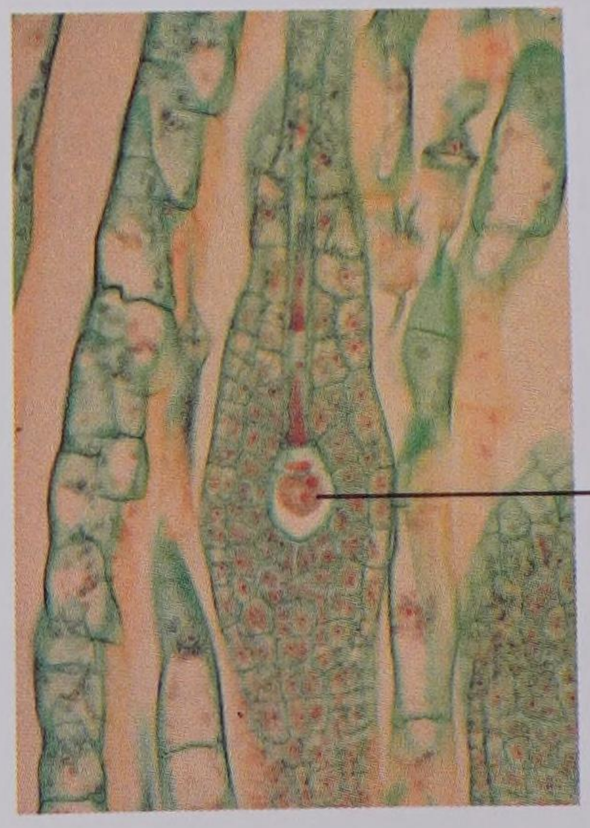


Figure 15.4 Moss antheridia.

Ⓢ 6. Find the *antheridia* (fig. 15.4) at the top of the male shoot. These are filled with many flagellated *sperm.* When *sperm* produced by the antheridia swim in a film of water to the *egg* in the archegonia, a *zygote* results. This is the beginning of the *sporophyte* generation.

Sporophyte Generation

Ⓢ 1. Examine the living sporophyte of a moss specimen provided, a moss in a moss minimarsh, or a plastomount of a shoot with sporophyte generation attached.

In the moss *Mnium,* the sporophyte generation is dependent on the female shoot. Why female? _____

_____

Ⓢ 2. Bracket and *label* the female gametophyte shoot and sporophyte generation in figure 15.5*a*.

Which of these generations is haploid? _____

Which is diploid? _____

Ⓢ 3. Examine a slide of a longitudinal section through the sporophyte generation of the moss (fig. 15.5*b*).

Ⓢ 4. Identify the stalk and sporangium. What is being produced in the sporangium? _____

By what process? _____

5. Examine a moss minimarsh (if available) carefully and see if you can observe the *protonema,* an algae-like structure, that develops from the germinating spores into the new gametophyte. A prepared slide of moss protonema may also be available to examine.

When spores germinate, what generation begins to develop? _____

Why is it proper to say that, in the moss, spores disperse the species? _____

By what means are spores disseminated? _____

Adaptation

Place an X by each characteristic that is an indication that mosses are likely to be found only in moist environments.

Characteristics

1. Lack of vascular tissue _____
2. Body covered by a cuticle _____
3. Swimming sperm _____
4. Egg and embryo protected by female shoot _____
5. Spores windblown _____

Figure 15.5 Moss sporophyte generation. ***a.*** Mature sporophyte generation attached to gametophyte generation. ***b.*** Longitudinal section through capsule showing location of spores.

Vascular Plants

Vascular plants include all the other plants to be studied in this laboratory and the next. The ones studied in this laboratory exercise are the *nonseed vascular* plants, while those studied in the next are the seed vascular plants. The nonseed vascular plants are better adapted to a dry environment than the bryophytes because the dominant sporophyte generation possesses vascular tissue. But the spores, which disperse the species in nonseed vascular plants, develop into an independent gametophyte generation that is water dependent because it lacks vascular tissue and also because it produces swimming sperm. The sperm must swim from the antheridia to the archegonia, where the eggs are produced. The zygote, protected from drying out within an archegonium, develops directly into the sporophyte generation.

Primitive Vascular Plants

The first 3 groups of vascular plants to evolve contain the *psilopsids,* the *horsetails,* and the *club mosses,* plants that are today greatly overshadowed by other higher vascular plants. But during the Carboniferous period the horsetails, club mosses, and *ferns* (also a nonseed vascular plant) grew large and abundant and later formed the great coal deposits that we still depend on today.

The gametophytes of these plants resemble those of ferns and will not be studied; we will limit our examination to the sporophytes.

Psilotum (Division Psilophyta)

Examine a preserved specimen of *Psilotum.* Note the horizontal underground stem, called a *rhizome,* the upright *aerial stems* and the dichotomous branch patterns, and the *bulbous sporangia* (fig. 15.6*a*). Traditionally, *Psilotum* was considered to be ancestral to all the other tracheophytes. This assumption is being questioned, and now many consider *Psilotum* to be a primitive type of fern only.

Horsetail (Division Sphenophyta)

1. Examine the underground stems (rhizomes) of *Equisetum,* some of which have *vegetative* shoots and some of which have *spore-bearing* shoots attached to them.
2. Note the minute, scalelike leaves (fig. 15.6*b*).
3. Feel the stems. *Equisetum* contains a large amount of silica in its stem, which helped give these plants the name "scouring rushes."
4. Examine a spore-bearing (fertile) shoot. Note the spore-bearing portion at the top. The entire spore-bearing tip is called a *strobilus,* and it contains *sporangiophores* (structures bearing sporangia).
5. Remove a single *sporangiophore* and examine it using a binocular dissecting microscope or a hand lens. There are spores inside each sporangiophore.

Club Moss (Division Lycophyta)

1. Examine a living or preserved specimen of *Lycopodium.*
2. Note the shape and size of the leaves and the method of branching of the stems (fig. 15.6*c*).
3. Note the strobili (plural of strobilus), which are composed of spore-bearing leaves called *sporophylls.*
4. Remove a single *sporophyll,* make a wet mount (omit the cover glass), and examine it using a binocular dissecting microscope or a hand lens.

Fern (Division Pterophyta)

Sporophyte Generation

1. Study the diagram of the life cycle of the fern (fig. 15.7) and find the *sporophyte* generation. This large, complexly divided leaf is known as a *frond.* Fronds arise from an underground stem called a *rhizome.*
2. Examine a living or preserved specimen of a frond, and on the underside notice a brownish clump called a sorus (pl. sori), each one a cluster of many sporangia (fig. 15.8).

   What is being produced in the sporangia? _____

   Considering the fact that it is this generation that we call the fern, what generation is dominant in ferns?

   _____
3. Examine a prepared slide called young *sporangia,* cross section (fig. 15.9).
4. Study the slide carefully and locate the fern *leaf* above and the *sorus* below.
5. Within the sorus find the sporangia and spores. Notice the *indusium* (not present in all species), a shelflike structure that protects the sporangia until they are mature.

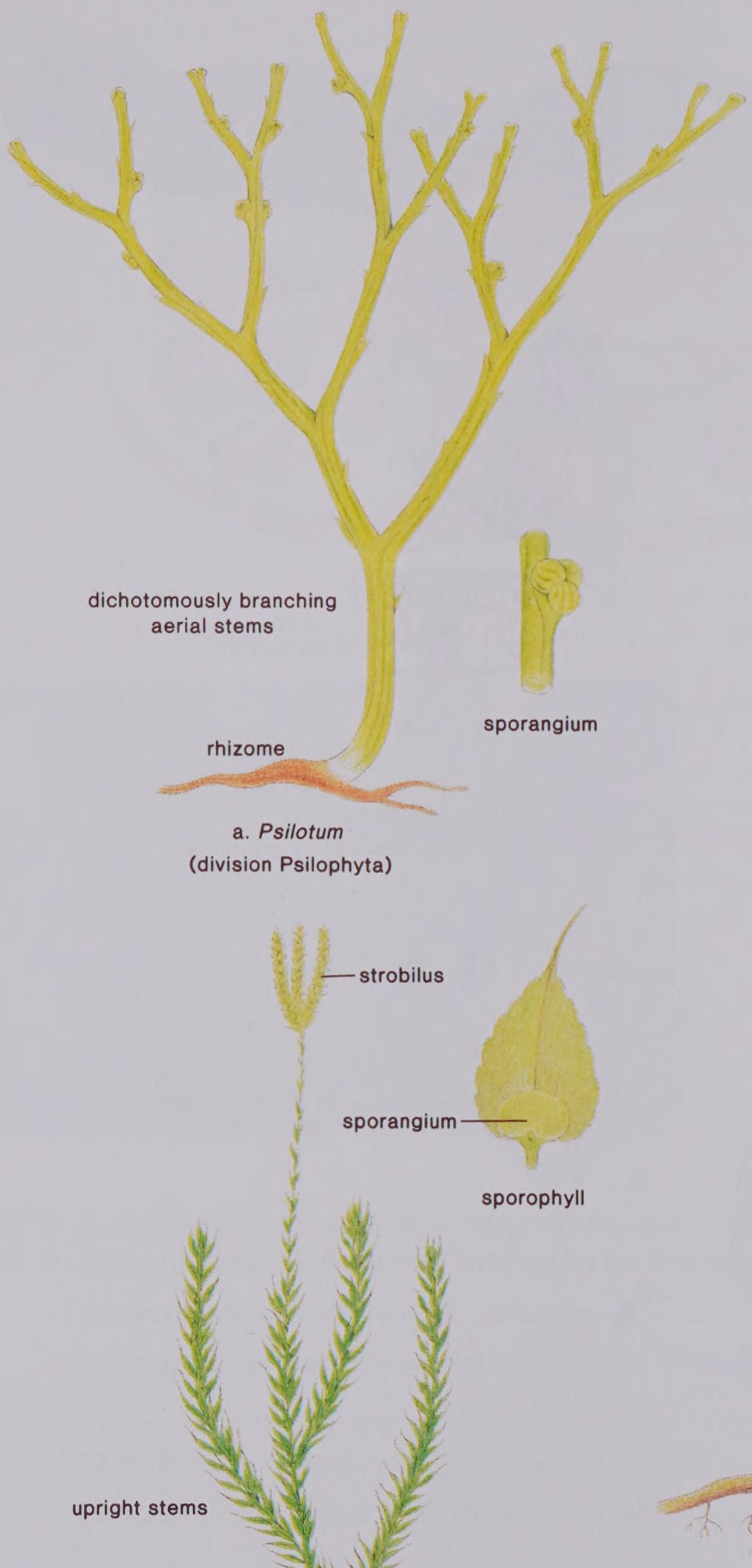
Gametophyte Generation

1. Examine a plastomount showing the fern life cycle.
2. Notice a small, heart-shaped structure, the *prothallus.*
   Can you find this structure in your fern minimarsh (if available)? The prothallus is the gametophyte generation of the fern. Most persons do not realize that this structure exists as a part of the fern life cycle.

   What is the function of this structure? _____

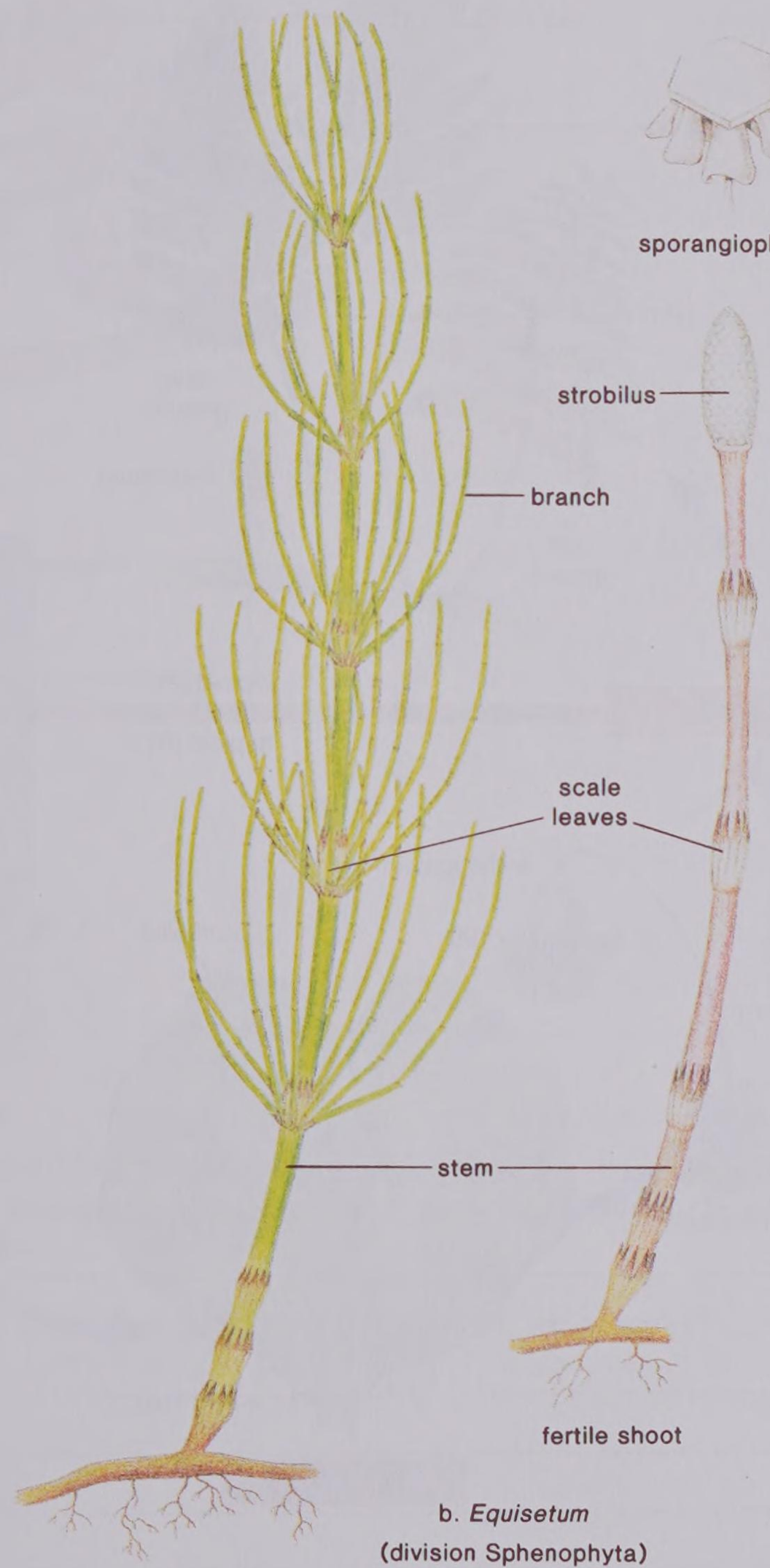
   _____

Figure 15.6 Primitive vascular plants.



a. *Psilotum*
(division Psilophyta)

c. *Lycopodium*
(division Lycophyta)



b. *Equisetum*
(division Sphenophyta)

Figure 15.7 Life cycle of a fern.

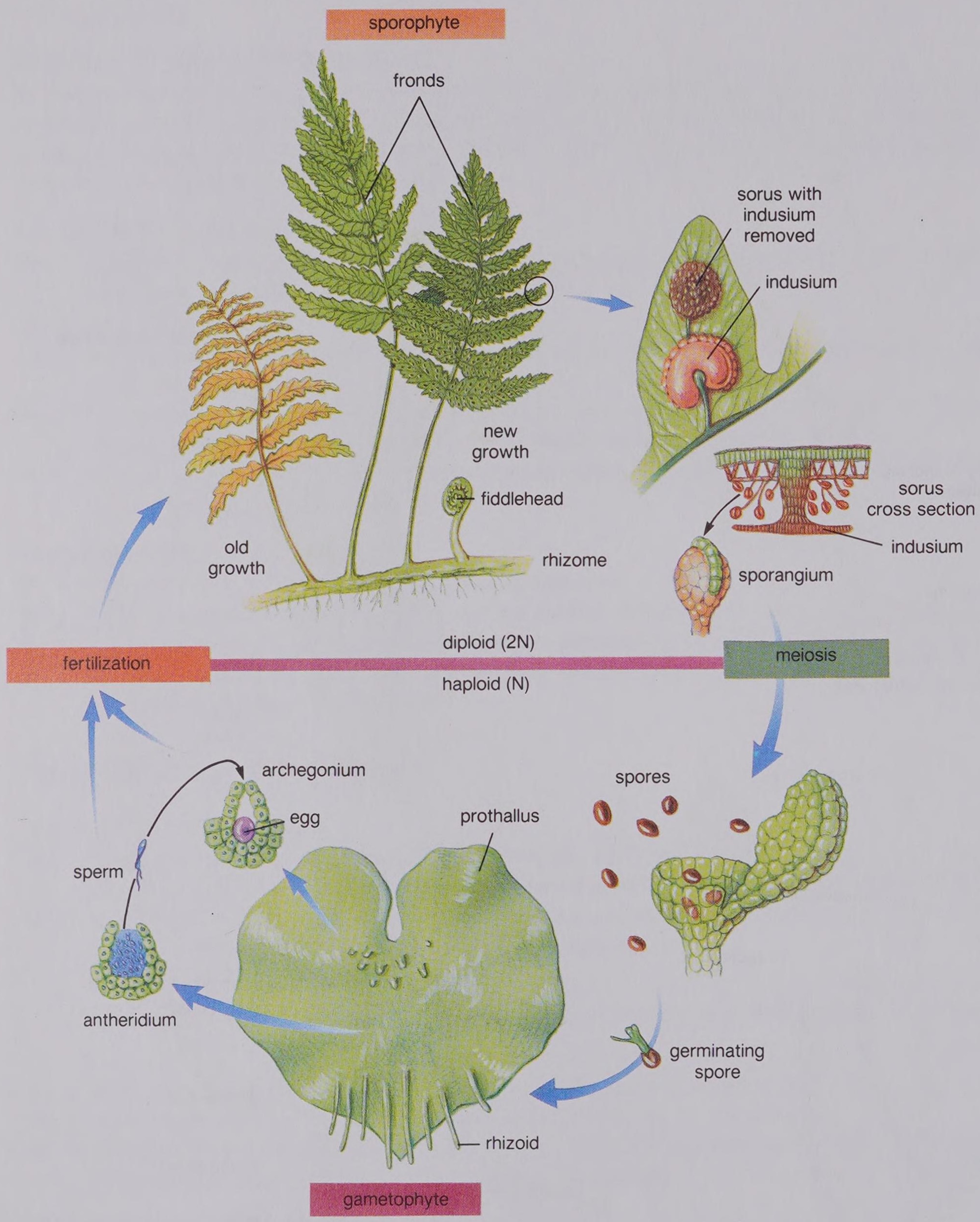


Figure 15.8 Cross section of a frond leaflet showing internal anatomy of a sorus.

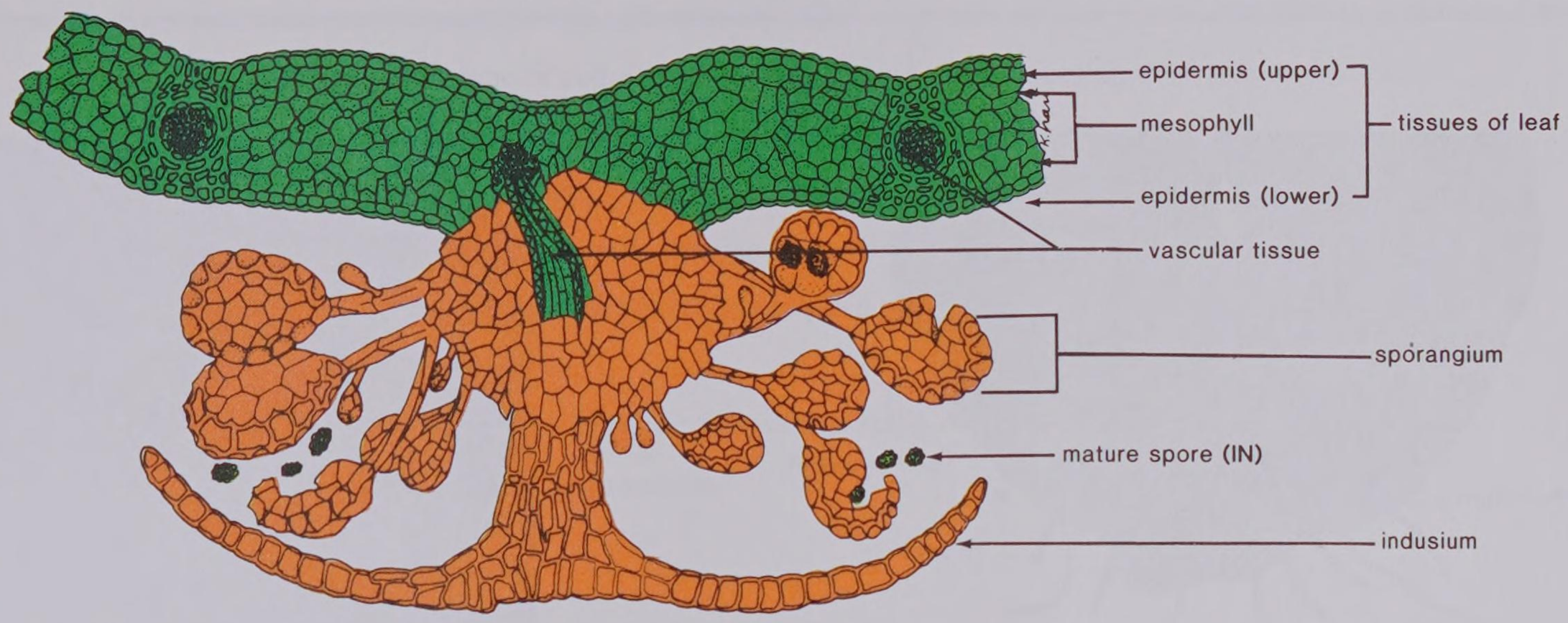


Figure 15.9 Underside of frond leaflets showing sori.

S 3. Examine whole mount slides of fern prothallium-archegonia and fern prothallium-antheridia (fig. 15.10).

S 4. If you focus up and down very carefully on the archegonia, you may be able to see an egg inside. What is being produced inside the antheridia? _____

When sperm, produced by the antheridia, swim to the archegonia in a film of water, what results? _____

_____

The latter develops into what generation? _____

How are ferns dispersed from one area to another? _____

_____

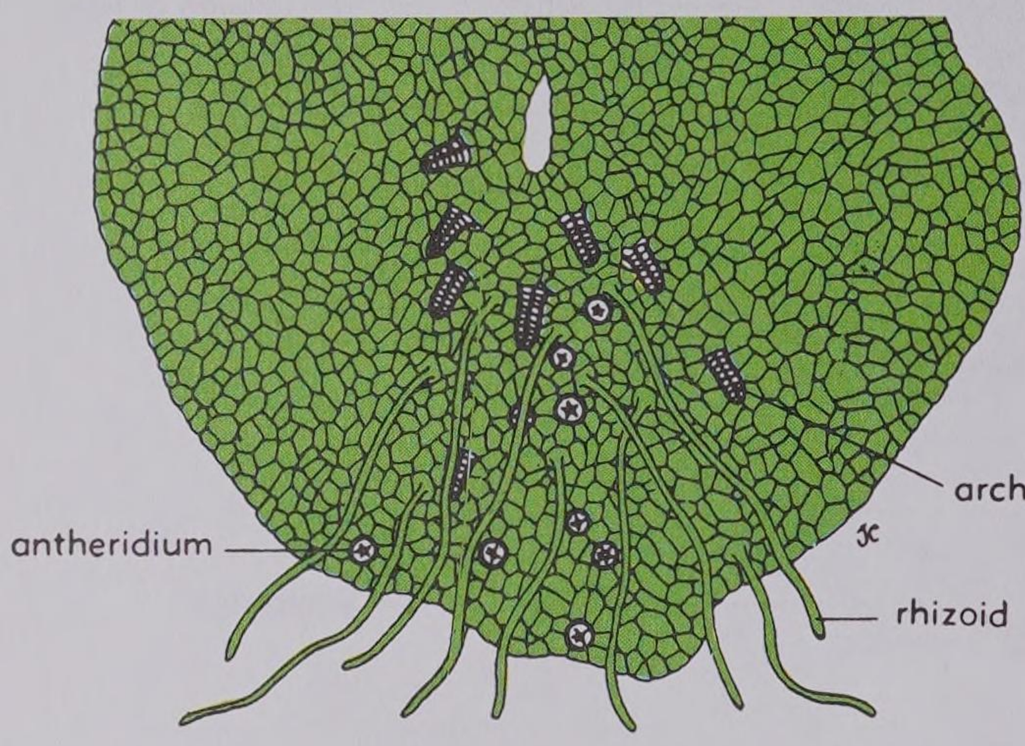
Is either generation in the fern dependent for any length of time on the other generation? _____

Adaptation

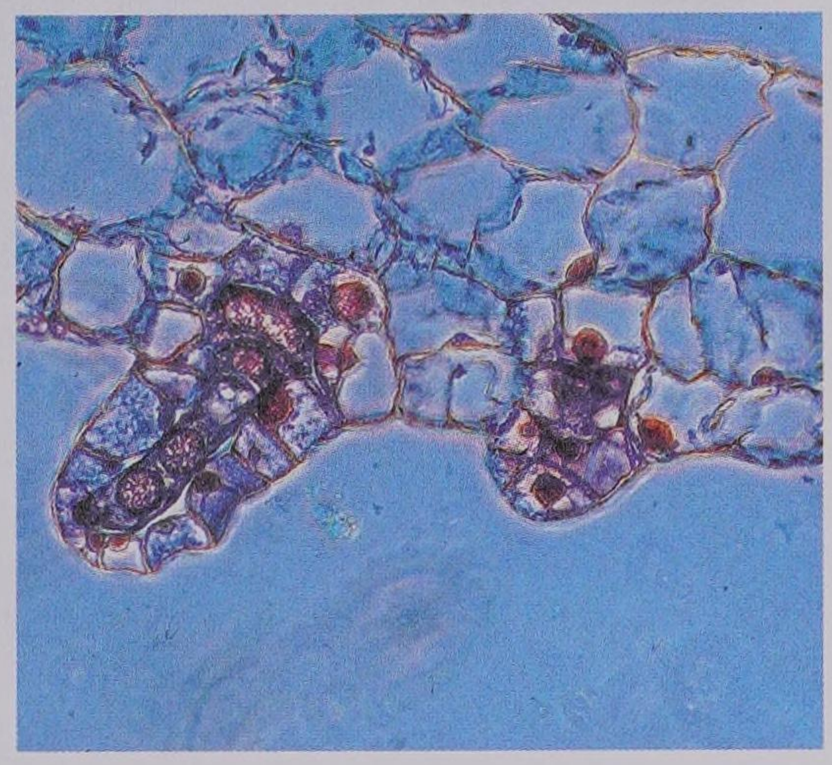
S 1. Identify one way in which the fern is adapted to a dry environment compared to the moss. _____

S 2. Give one characteristic of the fern that would indicate that a fern requires outside water for sexual reproduction to occur. _____

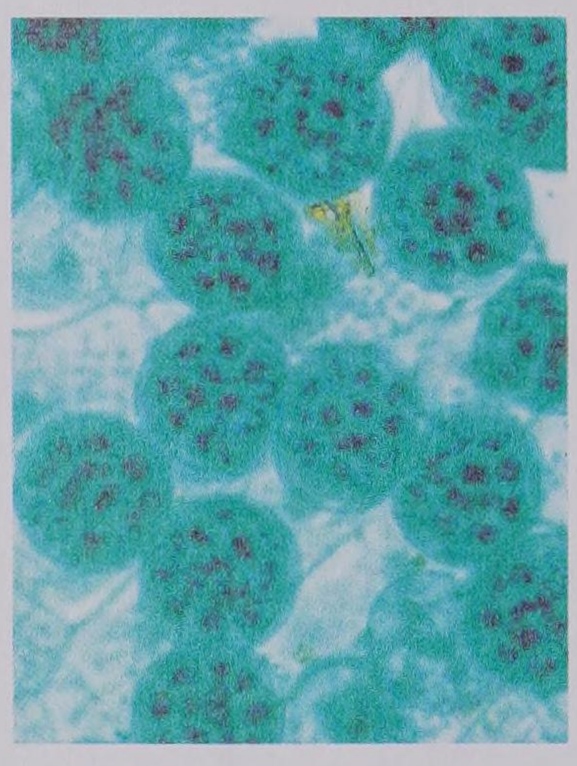
Figure 15.10 Fern gametophyte. ***a.*** Diagram. ***b.*** Archegonia. ***c.*** Antheridia.



a.



b.



c.

Summary

Complete table 15.2.

Table 15.2 Comparison of Nonseed Plants

	Dominant Generation	Vascular Tissue (present or absent)	Swimming Sperm (present or absent)	Dispersal of Species by
Moss				
Fern				

Laboratory Review 15

_____ 1. What type of cell division occurs during the production of spores in mosses?

_____ 2. Why do the bryophytes require external water for reproduction?

_____ 3. In a moss plant, which generation is the dominant one?

_____ Which generation is the dependent one?

_____ 4. Is the sporophyte generation haploid or diploid?

_____ 5. What is a frond?

_____ 6. Is the prothallus of the fern nutritionally dependent or independent?

7. What are 2 major ways ferns differ from mosses?

Laboratory

16

Diversity III: Seed Plants

Learning Objectives

Students should be able to

1. discuss how life cycles differ between nonseed and seed plants;
2. contrast and compare the 4 types of gymnosperms;
3. identify the sporophyte and gametophyte generations of the pine tree and flowering plants;
4. discuss in what manner both the pine and flowering plant are fully adapted to life on land;
5. identify the fruits studied in this laboratory;
6. compare the bean seed to the corn kernel and compare the process of germination in these 2 types of plants.

Introduction

In this laboratory we study those vascular plants that disperse the species by means of *seeds*. Seed plants fulfill most of the criteria, listed in table 15.1, necessary for successful life on land. The dominant sporophyte generation contains vascular tissue, which not only transports water but also serves as an internal skeleton, allowing these plants to oppose the force of gravity. For example, all trees are seed plants. The sporophyte (2N) of vascular plants is the majority of the plant body and includes the roots, stems, and leaves and some parts of the flower. The sporophyte's roots allow it to obtain water from the environment, and a coating of protective material (cuticle) on the outside of the plant prevents uncontrolled water loss. Their method of reproducing, seed production, is not dependent upon water. To understand the mechanism that has eliminated water-dependent fertilization, it is necessary to study the life cycle of the seed plants (fig. 16.1). New features are introduced. There are 2 different types of sporangia and spores. Each *megasporangium* produces a *megaspore* that develops into a female gametophyte generation. The *microsporangia* produce *microspores,* each of which develops into a male gametophyte generation. The mature male gametophyte generation is the pollen grain. This structure replaces swimming sperm in the seed plants.

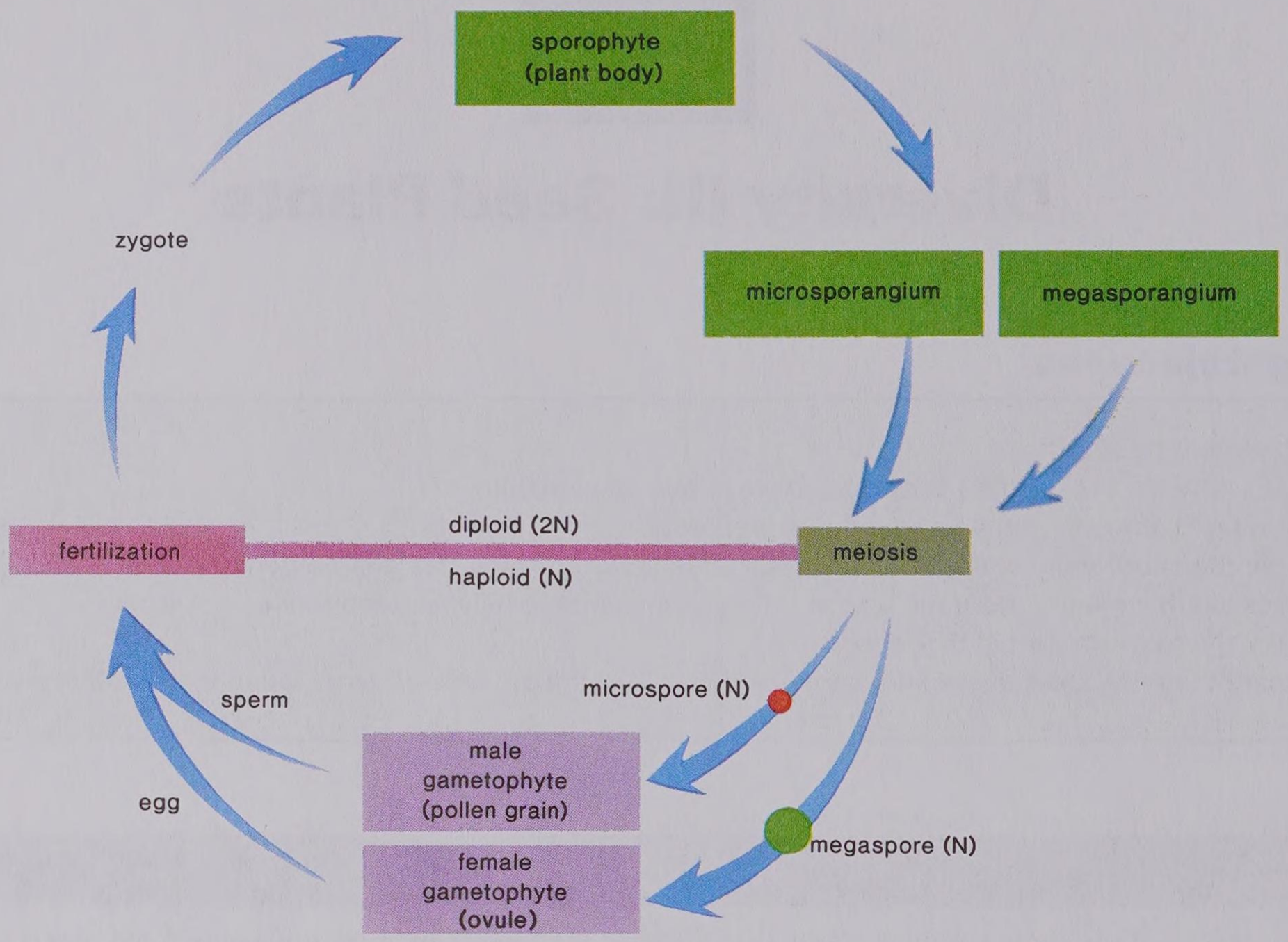
Gymnosperms

There are 4 groups of plants that are termed gymnosperms. Of these, the conifers represented by the *pine tree* are studied in detail; however, the laboratory may have representative specimens of the other 3 divisions for you to examine.

Cycads (division Cycadophyta) are cone-bearing, palmlike plants found today mainly in tropical and subtropical regions (fig. 16.2*a*). The fossil record indicates that cycads were quite numerous during the Age of the Amphibians, 350 million years ago. In the cycads, there are separate male and female plants. If you are examining *Zamia,* note that there are male and female cones. If you are examining *Cycas,* note that only the male plant bears cones; the female reproductive structure is much more leaflike.

Only one species of *ginkgos* (division Ginkgophyta), the maidenhair tree (fig. 16.2*b*), survives today. The maidenhair tree was largely restricted to ornamental gardens in China until it was discovered that the tree, unlike others, does quite well in polluted areas. Since the female trees produce rather smelly seeds, it is the custom to use only male trees in city parks. The maidenhair tree is recognized by its fan-shaped leaves with a forked vein pattern.

Figure 16.1 Life cycle of a seed plant.



The *gnetophytes* (division Gnetophyta) include 3 genera of plants that look quite different from one another. *Ephedra* is the genus that occurs in the United States (fig. 16.2*c*). These plants, which are many-branched shrubs with small, scalelike leaves, are found largely in desert regions.

Ⓢ If possible, *examine* the reproductive structures of *Ephedra*. Do they resemble flowers? _____ The vascular tissue of gnetophytes also resembles that of the angiosperms, and many botanists believe that the gnetophytes are closely related to flowering plants.

The *conifers* (division Coniferophyta) are by far the largest group of gymnosperms. The pine has been chosen as representative of these plants (fig. 16.2*d*).

Pine Life Cycle

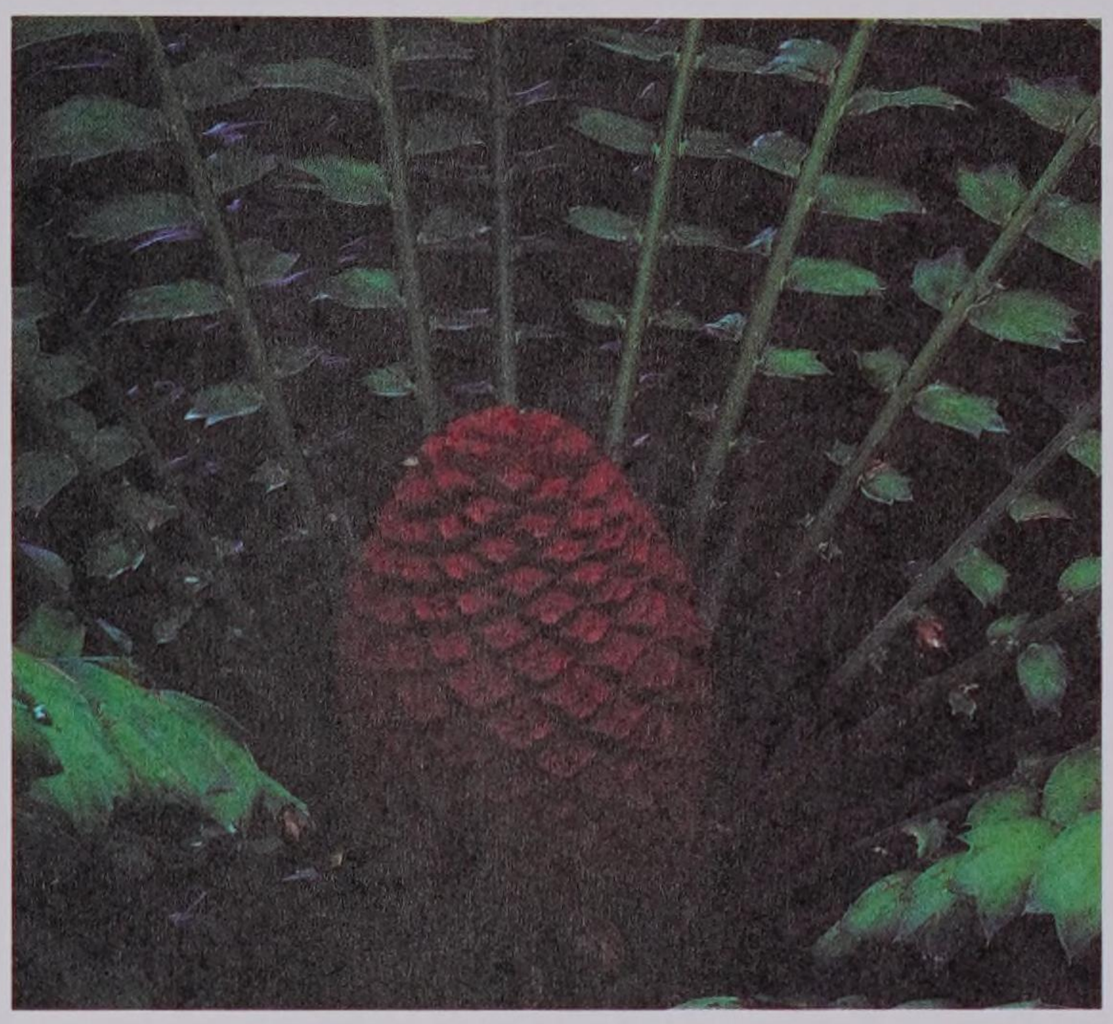
Ⓢ Study the diagram of the life cycle of the pine and find the *sporophyte* generation (fig. 16.3). This generation is the obvious plant (stem, leaves, roots, cones [part]). The gametophyte generation (male and female) are microscopic.

Cones

The pine tree bears cones (fig. 16.4).

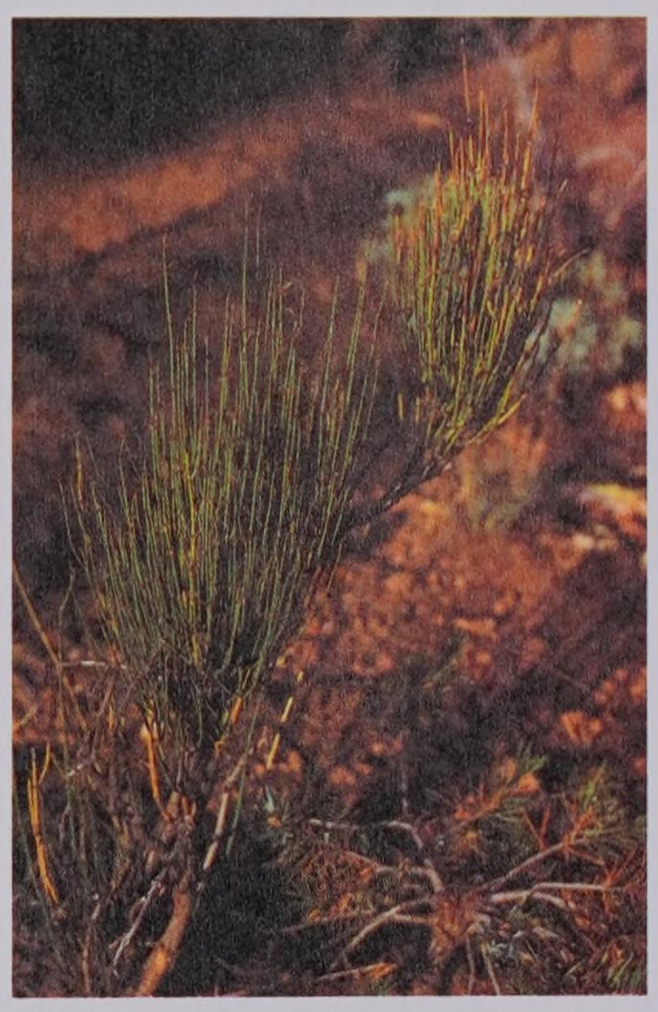
Ⓢ 1. Observe the male and female cones on display.
Ⓢ 2. Compare their relative sizes and structures.
Ⓢ 3. Remove a single scale (sporophyll) from the male cone and from the female cone, which has been heated so that the cone opens.
Ⓢ 4. Observe with a dissecting microscope the lower surface of each scale from the male cone.
Ⓢ 5. Note the 2 microsporangia on the lower surface of each scale of the male cone.
Ⓢ 6. On the upper surface of each scale of the female cone, note the 2 structures. Depending on the maturity of the cone, these structures may still be *ovules* or may be the *seeds* that develop from them after fertilization. These structures held the megasporangium and later the female gametophyte generation.

Figure 16.2 Gymnosperms. There are 4 divisions of gymnosperm plants. ***a.*** Cycads (division Cycadophyta). ***b.*** Ginkgo (division Ginkgo phyta). ***c.*** Gnetophytes (division Gnetophyta). ***d.*** Conifers (division Coniferophyta).



a.

b.



c.

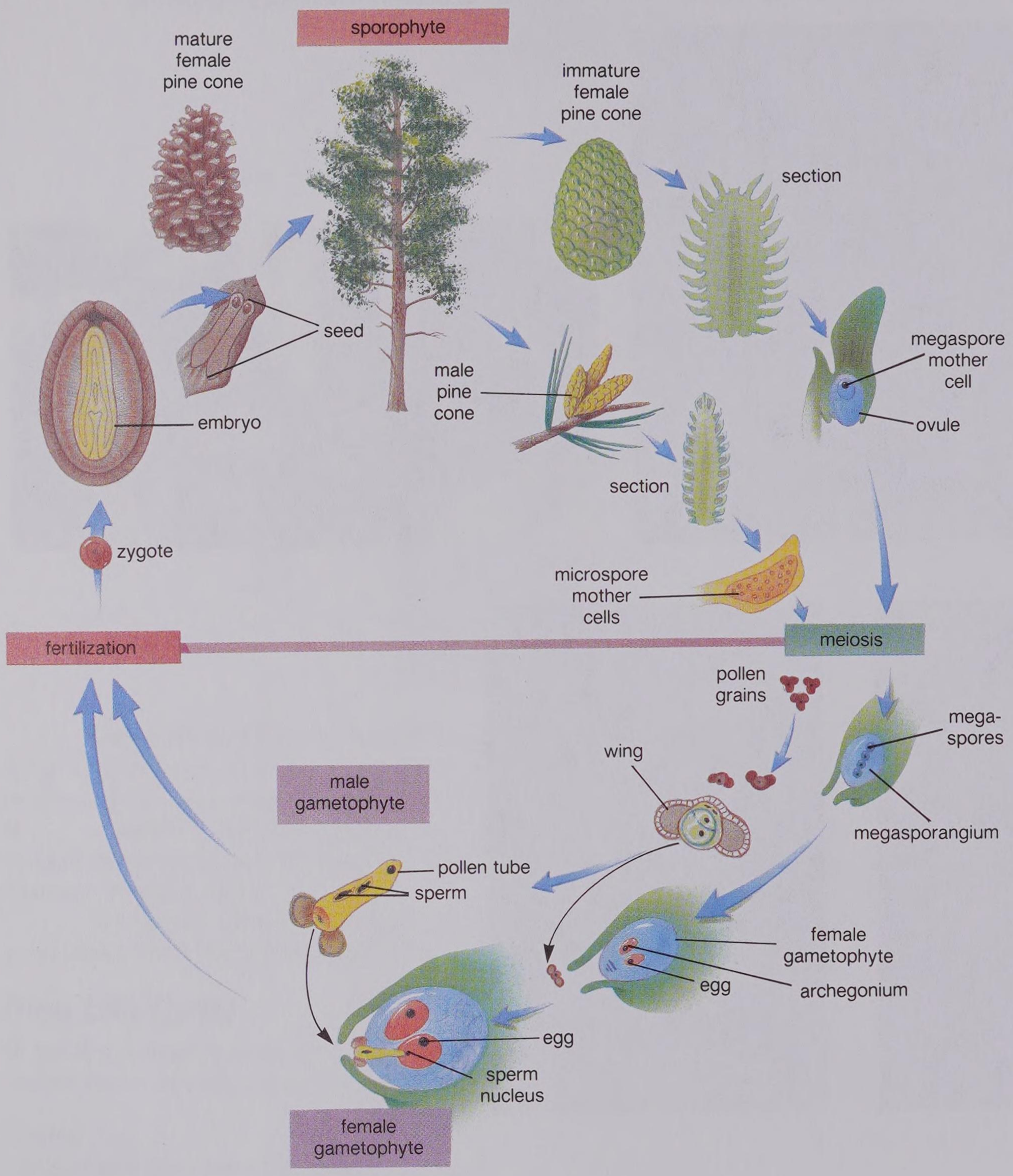
d.

Slide Study

Staminate (Male) Cone

1. Examine the prepared slide of a longitudinal section through a mature male pine cone and figure 16.5. On the lower surface of each scale are the *microsporangia,* in which the microspore *mother cells* produce *microspores.* Each microspore develops into a *pollen grain.*
2. Under high power, focus on a pollen grain and note the external *wings* and interior *cells*. One of these cells will divide to produce nuclei, one of which becomes the *sperm* nucleus which functions to fertilize the egg after pollination. The other cell forms the pollen *tube.*

Figure 16.3 Life cycle of a pine.



Ovulate Cone

In the female cone (fig. 16.6), 2 ovules develop on the upper surface of each scale. An ovule consists of a megasporangium surrounded by protective layers. The ovule is not released from the cone but remains within the tissues of the parent sporophyte. Within the megasporangium, the megaspore mother cell undergoes meiosis to produce 4 megaspores, 3 of which disintegrate.

Ⓢ Examine the prepared slide of a longitudinal section through an ovulate (female) cone. In some ovules, you will see a *megaspore mother cell* surrounded by *nutritive cells*. You may also be able to find *pollen grains* just outside or within the integuments of an ovule. At about the time the megaspore mother cell is undergoing meiosis, the scales swell and open to allow the wind-dispersed pollen grains to enter. *Pollination* is the transfer of pollen from the male to the female cone.

Figure 16.4 Pine cones.

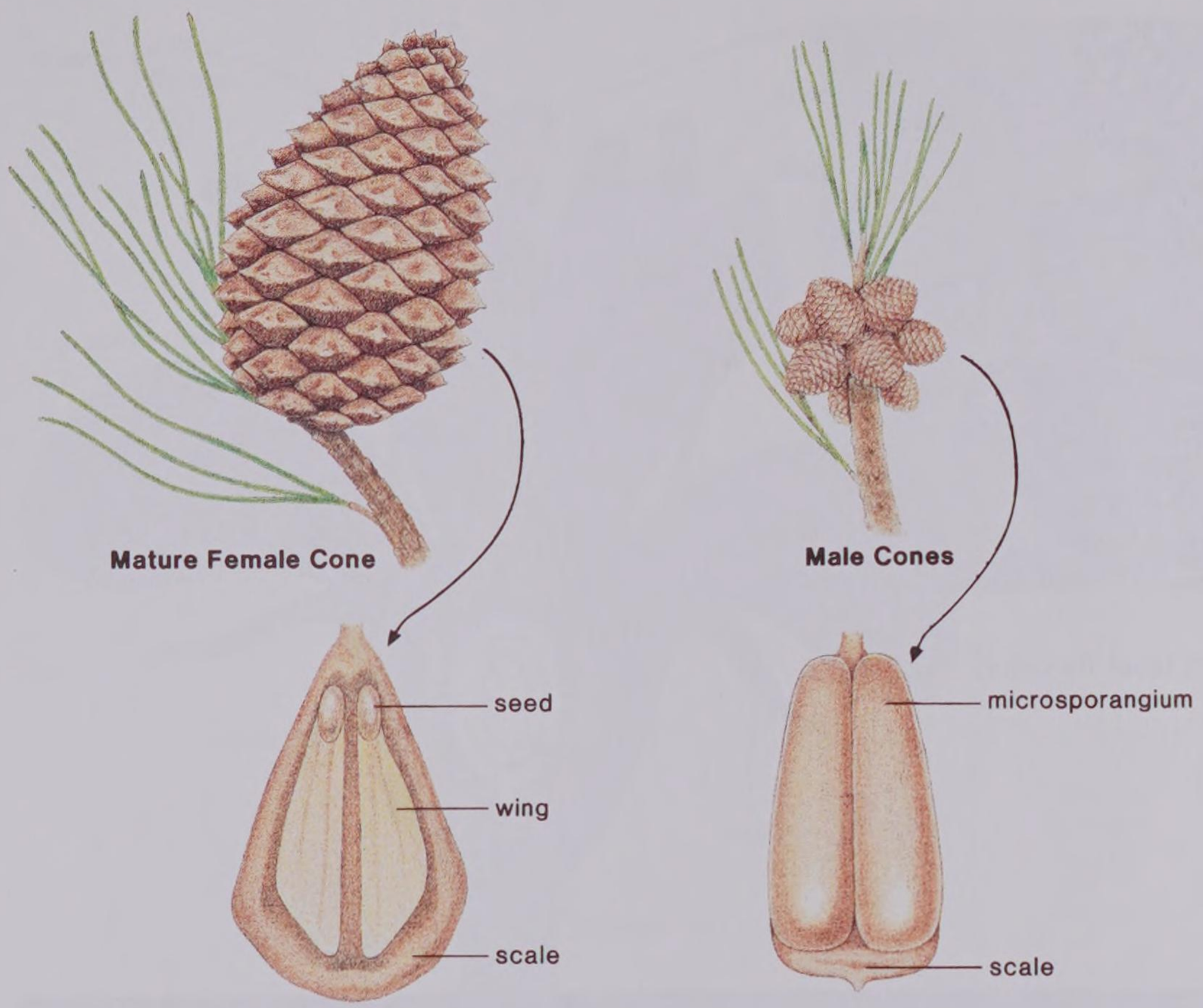
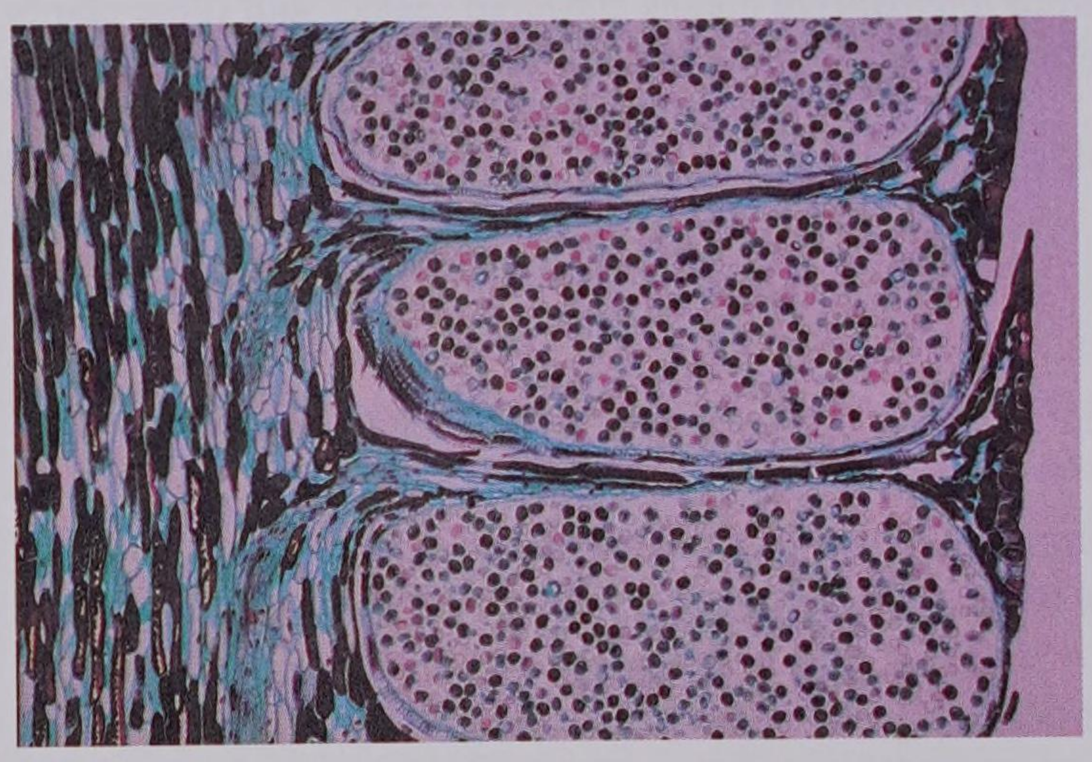
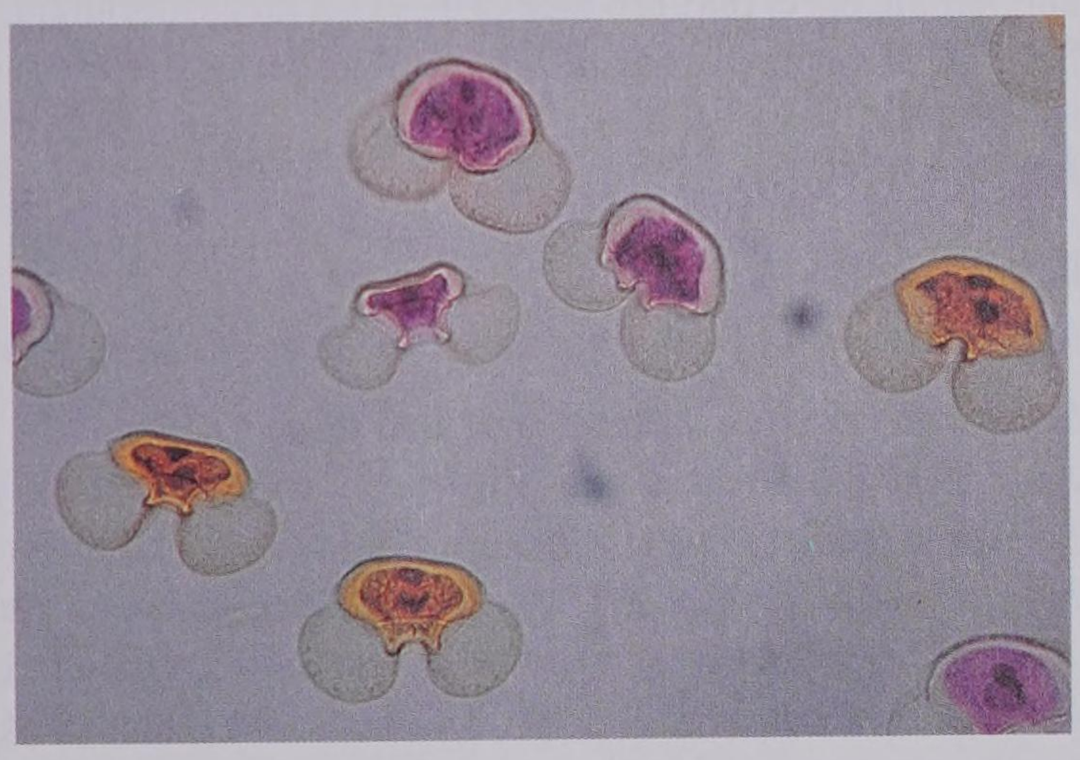


Figure 16.5 Photomicrograph of pollen grains. ***a.*** Longitudinal section through male pine cone showing pollen grains within microsporangia. ***b.*** Enlargement of pollen grains.



a.



b.

The megaspore undergoes a series of mitotic divisions and develops into the female gametophyte that contains 2 archegonia, each of which encloses a single large egg. What generation is now present? _____ A zygote is formed when a sperm travels down a tube, called a pollen tube, to fertilize this egg.

Seed

A seed contains the embryonic plant and nutrient material.

S 1. If available, examine pine seeds. How are they dispersed? _____
In seed plants, seeds disperse the species. The word *gymnosperm* means "naked seeds," and the seeds themselves are not covered.

S 2. If directed to do so by your instructor, *dissect* a seed, and with the help of a binocular dissecting microscope or hand lens, attempt to find the embryo.

Figure 16.6 Photomicrograph of longitudinal section of megasporangium. Note pollen grains near entrance.

S 3. *Sketch* the embryo's shape and label its color.

Angiosperms (Division Anthophyta)

The angiosperms are the *flowering plants*. The flower contains male parts that produce pollen in their microsporangia and female parts that produce ovules in a megasporangia. The word *angiosperm* means "covered seeds," and these plants have seeds enclosed in fruits. The angiosperms are the last group of plants to have evolved and are the most successful.

Flower

Model

Identify these parts of a flower using figure 16.7 as a guide.

Sepals: the outermost set of modified leaves, collectively termed the *calyx*. The sepals are green in most flowers.
Petals: the inner leaves that collectively constitute the *corolla*. The petals tend to be brightly colored.
Stamens: consists of a swollen terminal anther and a slender supporting filament; the anther releases pollen grains.
Pollen grain: the male gametophyte generation that develops a pollen tube. Observe demonstration-prepared slides of pollen grains that have germinated and have pollen tubes.
Pistil: consists of a swollen basal ovary, a long, slender style, and a terminal stigma.
Ovule: structure within the ovary that contains the megasporangium and develops into the seed.

Living Flower

S 1. Carefully inspect a living flower. What is the common name of your flower? _____

S 2. Remove the *sepals* and *petals* by breaking them off at the base. Are the stamens taller than the pistil? _____

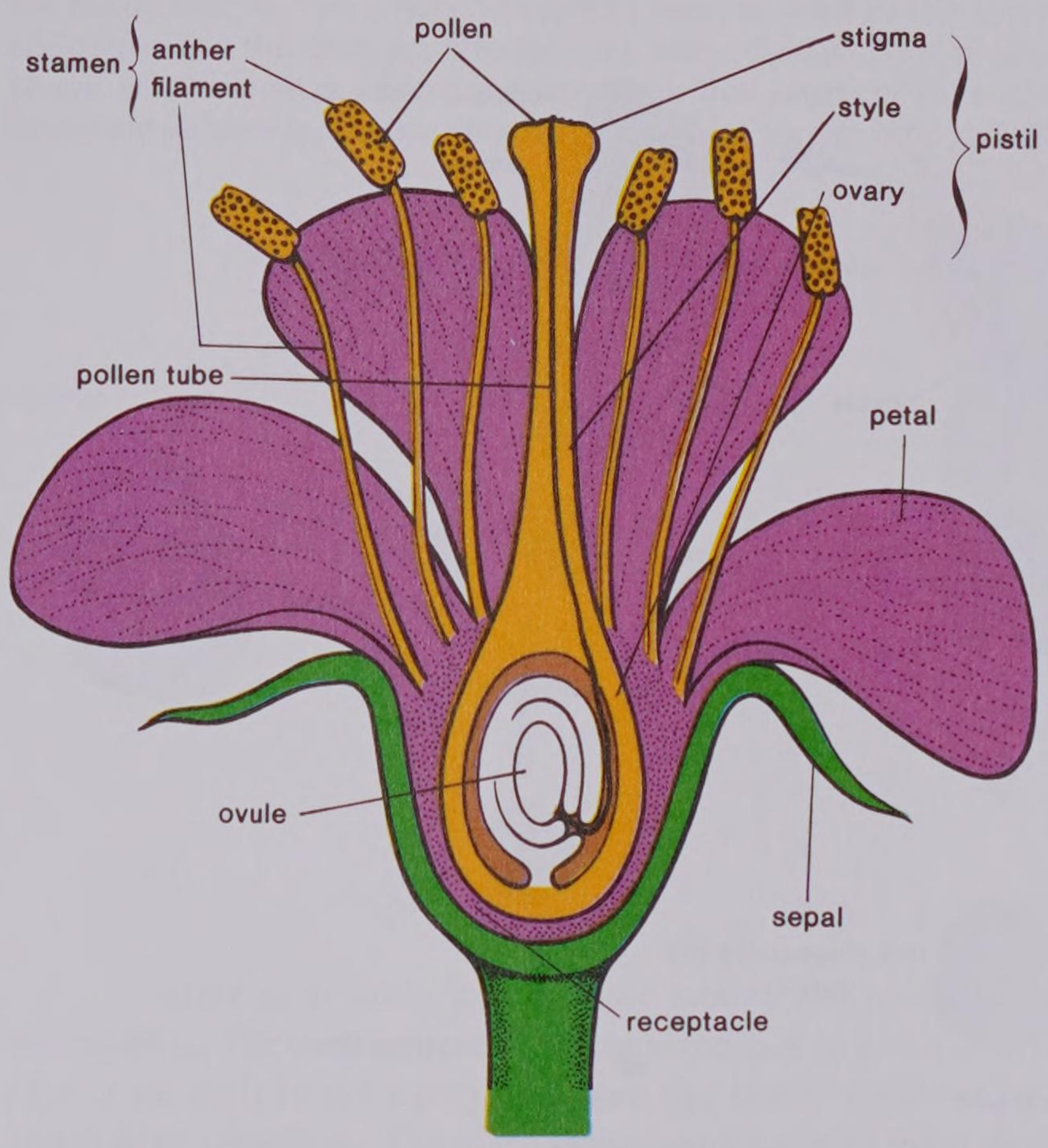
S 3. Remove a *stamen* and touch the anther to a drop of water on a slide. If nothing comes off in the water, crush the anther a little to squeeze out some of its contents.

S 4. Place a coverslip on the drop and observe with low and high powers. The somewhat spherical cells with thick walls are *pollen grains*.

S 5. Remove the *pistil* by cutting it free just below the base.

S 6. Make a series of thin cross sections through the ovary. Note that the ovary is hollow and that in this cavity there are small, nearly spherical bodies much larger than pollen grains. These are *ovules*.

Figure 16.7 Anatomy of typical flower.



Ⓢ 7. Remove an ovule that may be still attached by a stalk to the ovary wall. How many ovules are present in the entire ovary? _____

Ⓢ 8. Place the ovule on a drop of water on a slide, cover with a coverslip, and press firmly on the top of the coverslip with a clean eraser so as to smear the ovule into a thin mass.

Ⓢ 9. Observe with the microscope. You should be able to see some of the cells of which the ovule is composed.

Life Cycle of Flowering Plants

Ⓢ 1. Study the diagram of the life cycle of a flowering plant (fig. 16.8).

Ⓢ 2. Find the sporophyte (2N) generation. Notice that the sporophyte generation is the dominant generation, the one generally considered to be the plant. The gametophyte generation (male and female) is microscopic.

Ⓢ 3. Describe the sporophyte generation of a garden plant. _____
According to figure 16.8, each mother cell within the anther undergoes meiotic cell division to produce 4 haploid cells, the microspores. Each microspore nucleus divides mitotically to give a male gametophyte generation that matures into a pollen grain. Following pollination, the transfer of pollen from the anther to the stigma, a pollen grain germinates and produces a long pollen tube in which sperm travel to reach the ovule.

Ⓢ 4. Examine a prepared slide of *germinated pollen* grain, and try to identify the 3 nuclei within the pollen tube. One of these is the *tube* nucleus, and the other 2 are *sperm* nuclei. The germinated pollen grain is the mature male gametophyte generation.

Ⓢ 5. Sketch the appearance of the germinated pollen, and label the *tube nucleus* and the 2 *sperm nuclei*.

Figure 16.8 Life cycle of flowering plants.

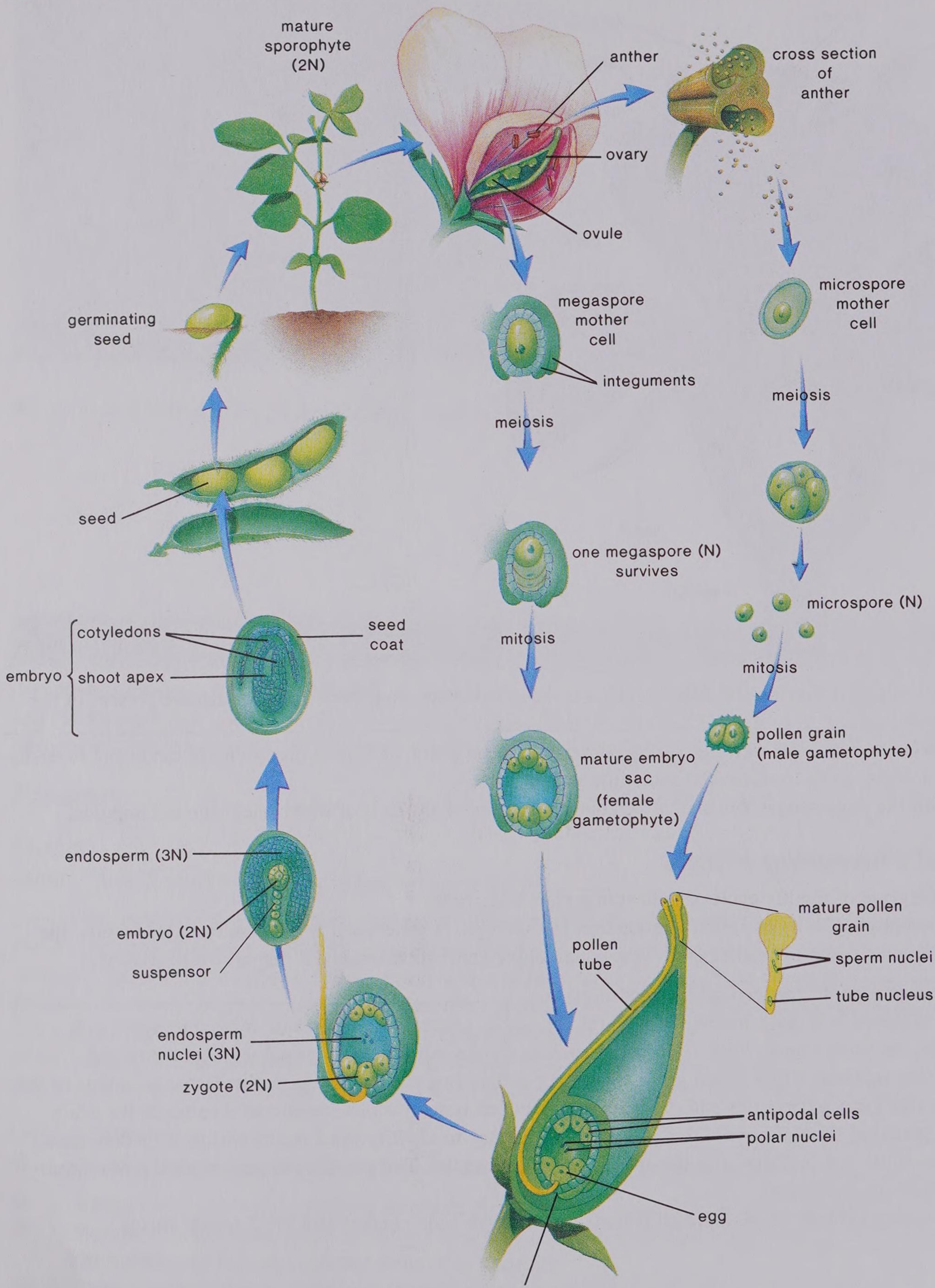
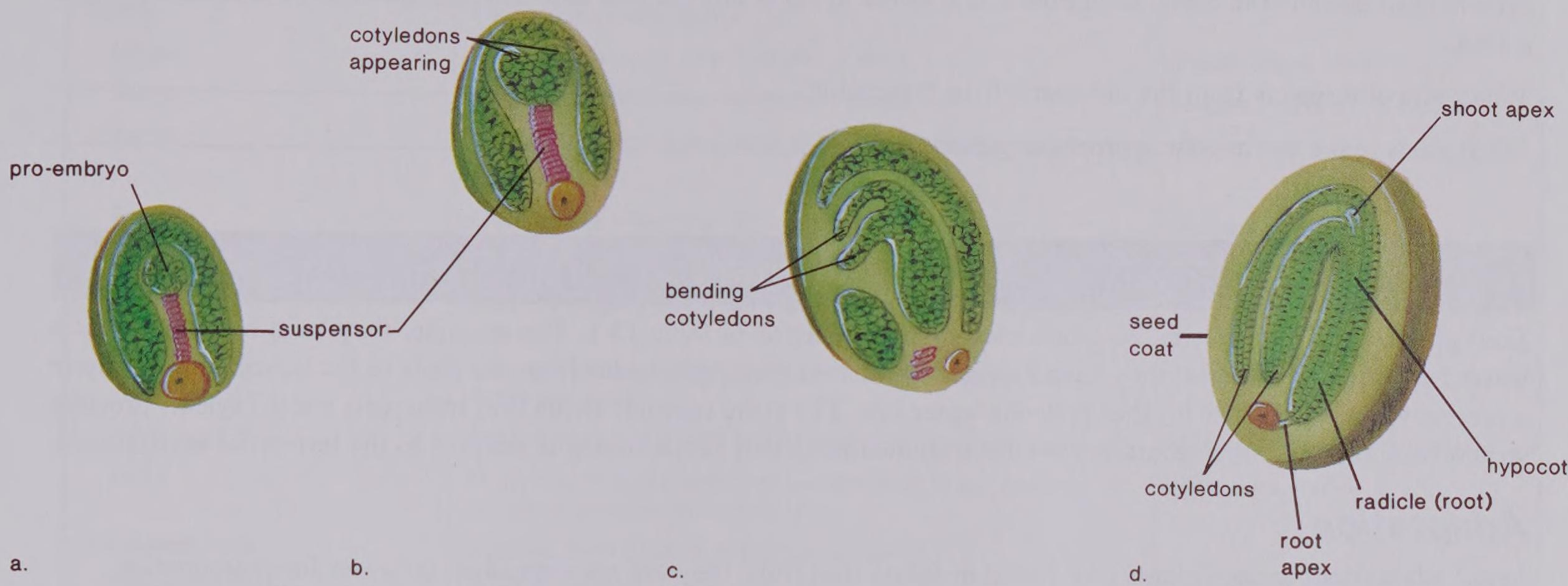


Figure 16.9 Stages in the development of a dicot embryo. ***a.*** The embryo itself is actually made up of the cells above the suspensor. ***b.*** The embryo becomes heart shaped as the cotyledons begin to appear. ***c.*** There is progressively less endosperm as the embryo differentiates and enlarges. As the cotyledons bend around, the embryo takes on a torpedo shape. ***d.*** The mature seed has a seed coat that protects the enclosed embryo. The embryo consists of the epicotyl, represented here by the shoot apex; the hypocotyl; and the radicle, which forms the first seedling root.



Inside each ovule, a megaspore mother cell nucleus undergoes meiosis to produce 4 megaspores, 3 of which disintegrate. The one remaining undergoes mitosis to give a multicelled *embryo sac,* the female gametophyte generation. One of the cells in the embryo sac is an egg that is fertilized by a sperm to give a zygote that will develop into a new sporophyte generation. The other sperm combines with polar nuclei to form a 3N nucleus that develops into *endosperm,* stored food. Now the ovule matures into a seed. A seed contains the embryonic sporophyte and stored food. Notice that the seeds of angiosperms develop within the ovary; this is why angiosperms have "covered seeds." The ovary becomes the fruit. All angiosperms produce fruit.

Development of the Embryo (Optional)

Four slides that show development of *Capsella,* a dicot plant, are available. Following fertilization, the zygote develops into an embryo within the embryo sac (female gametophyte) (fig. 16.8). With the help of figure 16.9, observe the following stages of development.

Globular Stage

The cells that will develop into the *embryo* are located above the *suspensor,* a chain of cells that attaches the embryo to the wall of the embryo sac. Surrounding the embryo is *endosperm* tissue, which will be used for the growth of the seed following germination. For now, the embryo receives nutrients from the previous sporophyte by way of the suspensor.

Heart-Shaped Stage

The *cotyledons,* or seed leaves (fig. 20.2), are appearing, and they form the lobes of the heart. Dicot seeds, such as *Capsella,* have 2 cotyledons, while monocots, such as corn, have one cotyledon. Often the cotyledons take up the endosperm tissue and serve as the immediate source of nutrients for the emerging seedling. You will look more closely at monocot and dicot cotyledons when you examine seeds later in this chapter.

Torpedo Stage

Now it is possible to distinguish the *shoot apex* and the *root apex* that contain apical meristem, the tissue that brings about the primary growth in a plant. The shoot apical meristem is responsible for above-ground growth, while the root is responsible for below-ground growth.

Mature Embryo

Now the embryo fills the space within the embryo sac, and the integuments of the ovule have become the *seed coat*. It is difficult to make out the *epicotyl,* a small portion of the embryo that lies above the attachment of the cotyledons. The epicotyl will become part of the stem. Beneath the cotyledons is the *hypocotyl* (will become lower portion of stem) and *radicle* (will become the root). Even now it is possible to make out the root cap. The mature embryo is enclosed within a seed.

What part of a seed is from the old sporophyte generation? _____

What parts make up the new sporophyte generation? _____

Summary

Seed plants fulfill the criteria for adaptation to land as listed in table 15.1. For example, they have roots that obtain water from the environment, they have vascular tissue that transports water from the roots to the leaves, and they have a protective covering (cuticle) that prevents water loss. The same vascular tissue that transports water (xylem) provides structural support. In this laboratory, we have studied how their reproduction is adapted to the terrestrial environment.

Adaptation

List 2 adaptations in seed plants (not found in ferns) that show they are not dependent on water for reproduction.

1. _____
2. _____

Comparison of Conifer to Flowering Plant

Fill in table 16.1.

Table 16.1 Comparison of Conifer to Flowering Plant

	Dominant Generation	Vascular Tissue (present or absent)	Dispersal of Species	Fruit (present or absent)
Conifer				
Flowering plant				

Fruits and Seeds

Fruits develop from the *ovary,* and *seeds* develop from the *ovules;* therefore, seeds are found within fruits. Table 16.2 lists the different kinds of fruits. There are 2 types of fruits (*simple* and *compound*), but in today's laboratory we will restrict ourselves to observing those that are simple.

To understand simple fruits, one must remember that they arise from either a simple ovary or a compound ovary. As the fruit develops from the ovary, the wall thickens, becoming the *pericarp*. The pericarp may be fleshy or dry. Examining the following fruits will help you gain a knowledge of fruit anatomy.

Fleshy Fruits

Peach: A peach is a drupe in which the fleshy pericarp surrounds a single seed. Is the entire pericarp fleshy? _____

Tomato: A tomato is a berry derived from a compound ovary containing many seeds. How many cavities can you make out? _____

Apple: An apple is a pome in which the bulk of the flesh comes from the enlarged receptacle that grows up and around the ovary. In an apple, the ovary largely consists of the core. Was the ovary simple or compound? _____

Table 16.2 Kinds of Fruits

Name	Description	Example
Simple Fruits	Develop from an individual ovary	
Fleshy	Pericarp is usually fleshy	
Drupe	From simple ovary with one pitted seed	Peach, plum, olive
Berry	From compound ovary with many seeds	Grapes, tomato
Pome	From compound ovary; flesh is from accessory flower parts	Apple, pear
Dry	Pericarp is dry	
Follicle	From simple ovary that splits open down one side	Milkweed, peony
Legume	From simple ovary that splits open along both sides	Peas, beans, lentils
Capsule	From compound ovary with capsules that split in various ways	Poppy
Achene	From simple ovary with one-seeded small fruit; pericarp easily removed	Sunflower
Nuts	From simple ovary with one-seeded fruit; hard pericarp	Acorns, hickory nuts, chestnuts
Grain	From simple ovary with one-seeded small fruits; pericarp completely united with seed coat	Rice, oats, barley
Compound Fruits	Develop from a group of individual ovaries	
Aggregate fruits	Ovaries are from a single flower	Blackberries, raspberries, strawberries
Multiple fruits	Ovaries are from separate flowers clustered together	Pineapple

Dry Fruits

Nuts: An acorn is a nut in which the pericarp is hard and thick. The cup is composed of fused bracts (modified leaflike structure). Many other things we commonly call nuts, such as walnuts and pecans, are actually other types of fruits. For example, what is a coconut? _____

Grain: A corn kernel is a grain in which the pericarp is tightly fused to the seed and cannot be separated from it.

Name some other grains: _____

Legume: A legume, such as the string bean, splits along 2 sides or seams.

Which part of a string bean is the fruit? _____

Which part are the seeds? _____

Another Legume

Ⓢ 1. Obtain a peanut and perform the following operations. Open the shell carefully.

What kind of a fruit is a peanut? _____

How do you know? _____

Ⓢ 2. Open an individual peanut carefully.

The meat of a peanut is the _____.

What do you find inside the peanut? _____

Is the peanut plant a dicot or monocot? _____

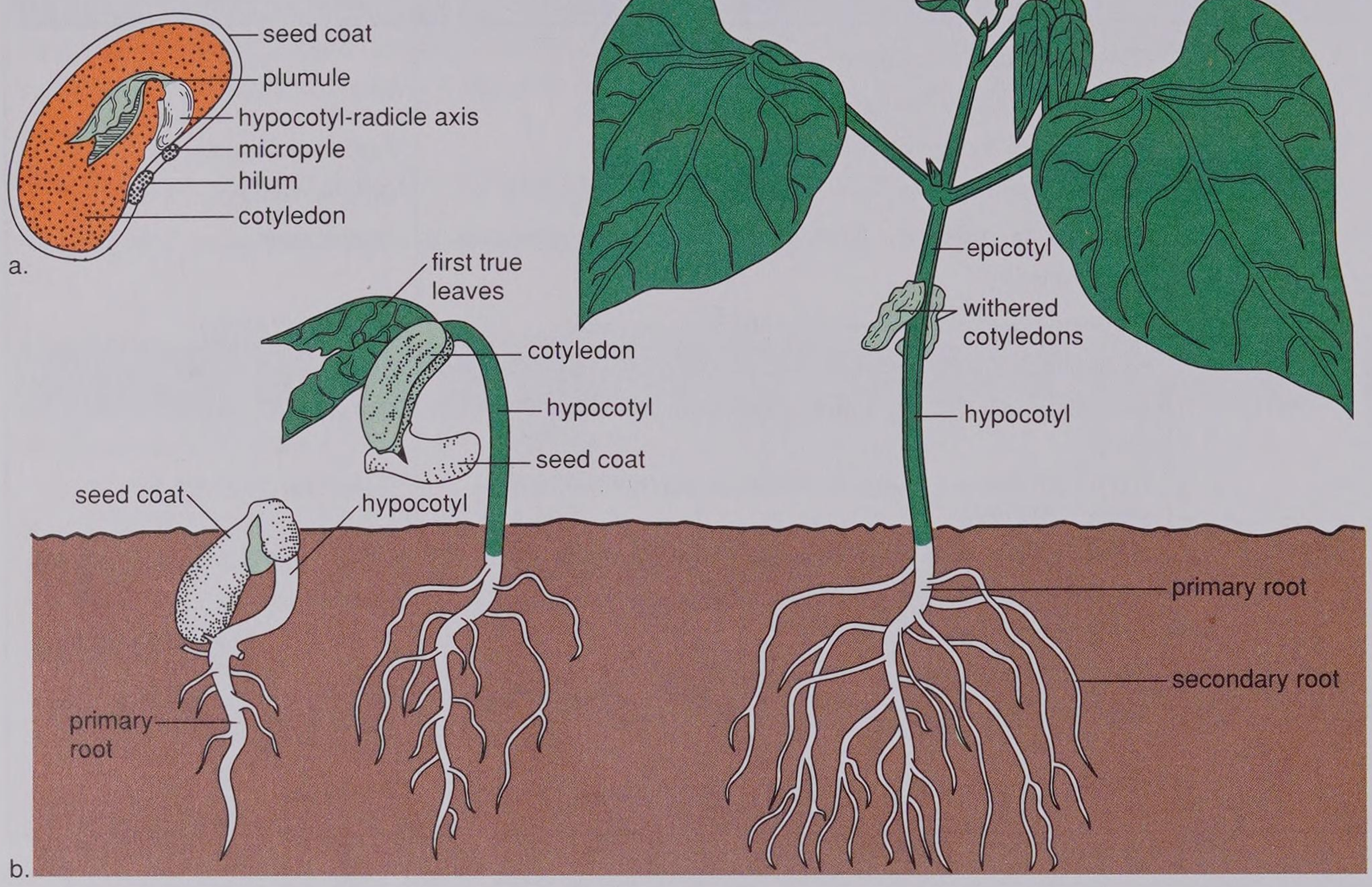
How do you know? _____

Seeds

You will look closely at the development of representative monocot and dicot seeds. Monocots have one cotyledon (seed leaves), and dicots have 2 cotyledons.

Ⓢ 1. Obtain a presoaked *bean* seed, a *dicot*. Identify, with the help of figure 16.10*a*, the *hilum*, a small white scar at which the ovule was attached to the ovary wall.

Figure 16.10 A common garden bean. ***a.*** Seed structure. ***b.*** Germination and development of the seedling.



S 2. Find the *micropyle,* where the pollen tube entered the ovule. Remove the covering from around the seed.

What is this covering? _____

Inside you find 2 halves to the bean seed. What is each of these? _____

What purpose do they serve? _____

What do you find lying between these halves? _____

With the help of figure 16.10, identify the *plumule, epicotyl, hypocotyl,* and *radicle.*

S 3. Obtain a *corn kernel,* a *monocot.* The covering of a corn kernel is the pericarp.

Should the kernel be called a seed or a fruit? _____

Why? _____

S With a razor blade, cut the kernel longitudinally as depicted in figure 16.11*a*.

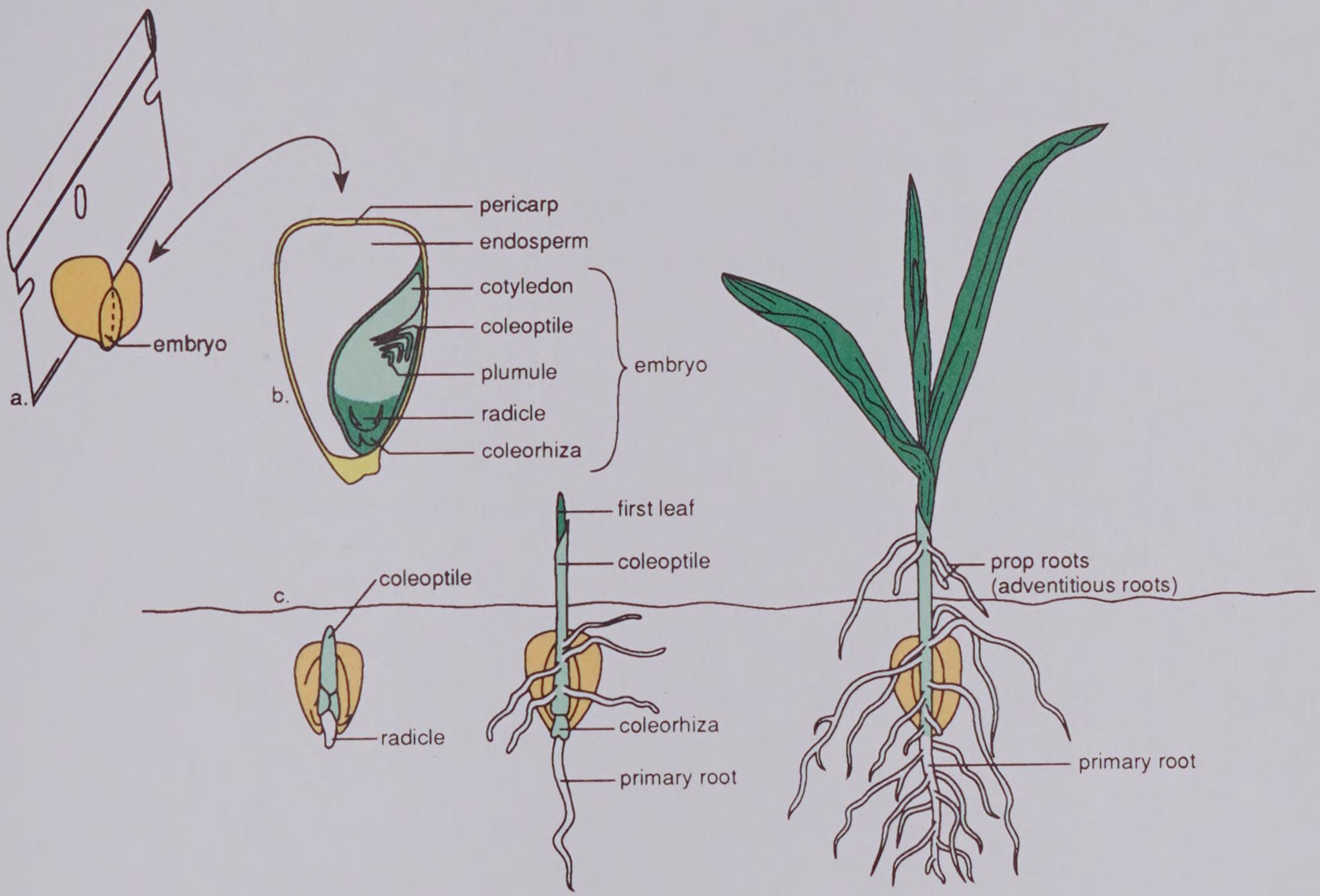
Is the corn plant a dicot or monocot? _____

How do you know? _____

Can you distinguish the endosperm from the cotyledon? _____

The endosperm is typically retained within monocot seeds but not dicot seeds.

Figure 16.11 Corn. ***a.*** Orientation of grain to obtain the proper section through embryo. ***b.*** Grain structure. ***c.*** Germination and development of the seedling.



Germination

Examine the stages of germinating *bean seedlings* in figure 16.10*c*.

What part of the bean seed germinates first? _____

The hypocotyl is the first part to emerge from the soil. What is the advantage of having the hypocotyl pull the plumule up out of the ground instead of pushing it up through the ground? _____

_____

Do the cotyledons stay beneath the ground? _____

Notice that in the corn seedling the emerging leaves are covered by a sheath called the *coleoptile* and that therefore they are not damaged as they push up through the earth (fig. 16.11*c*).

Does the cotyledon stay beneath the ground? _____

Laboratory Review 16

_____ 1. What is the dominant generation in both the gymnosperms and the angiosperms?

_____ 2. In seed plants, is the gametophyte dependent on or independent of the sporophyte generation?

_____ 3. How do seed plants disperse the species?

_____ 4. Is the pistil a male or female part of a flower?

_____ 5. What kind of tissue transports water and nutrients in seed plants?

6. How does pollen differ from sperm?

7. What is a major difference between gymnosperms and angiosperms in regard to their seeds?

8. What is the difference between pollination and fertilization?

Laboratory

17

Diversity IV: Primitive Invertebrates

Learning Objectives

Students should be able to

1. identify and classify all animals studied;
2. identify all slides studied and name distinguishing characteristics;
3. evaluate animals according to table 17.1.

Introduction

It is generally recognized that animals are organisms that

1. are multicellular;
2. are capable of locomotion or movement by means of contractile fibers;
3. have cells without a rigid cell wall and without chlorophyll;
4. are heterotrophic.

This is the first of 3 laboratories concerning the animal kingdom. Several slightly different evolutionary trees have been suggested for animals. One possible tree is given in figure 17.1. Today's laboratory concerns the first few phyla, which may be found on the "main trunk" of the tree. These animals are termed invertebrates because they lack the vertebral backbone found in vertebrates; they are also the primitive invertebrates because they evolved first.

The classification of animals is based in part on the criteria listed in table 17.1. In this laboratory session and the ones following, we will discuss each animal according to these criteria. The first criterion concerns *body plan.* A *sac* body plan is one in which the mouth is used both as an entrance for nutrient molecules and an exit for waste molecules. Animals with a mouth and an anus have the *tube-within-a-tube* body plan. The latter allows specialization of parts along the digestive canal to occur. The second criterion evaluates type of *symmetry. Radial* symmetry means that *any* longitudinal cut that passes through a midpoint will give 2 equal halves. Radial symmetry allows an animal to reach out in all directions. *Bilateral* symmetry means that only *one* cut through the midpoint gives 2 equal halves. Bilaterally symmetrical animals generally have well-developed head regions. The third criterion examines number and kind of *germ layers.* During the embryological development there may be only 2 germ layers—*ectoderm* and *endoderm*—or 3 germ layers—*ectoderm, endoderm,* and *mesoderm.* The presence of the mesoderm leads to the development of various internal organs. The fourth criterion evaluates the *level of organization.* Some animals do not have organs and instead have a *tissue* level of organization. Most animals have the *organ* system level of organization. The fifth criterion assesses the structure of the *coelom.* Some animals do not have a coelom, a central, fluid-filled body cavity lined by mesoderm in which the internal organs may be found; therefore, these animals are *acoelomate. Pseudocoelomates* have a body cavity incompletely lined by mesoderm and formed in a completely different manner from the true coelom cavity. *True coelomates* have a body cavity completely lined by mesoderm. The sixth criterion examines *segmentation.* When an animal is segmented (metamerism), it has *repeating parts,* such as our own vertebral backbone. The repeating series of parts makes it possible for some sections to become specialized for different functions in the advanced animals.

Figure 17.1 Evolutionary tree of animals. The terms on the trunk of this evolutionary tree are not taxonomic terms, but they are descriptions of various phases of embryonic development (i.e., deuterostomes) or their basic body plan (i.e., radiata, coelomate). If you are unfamiliar with the meaning of any of these terms, look them up in your textbook.

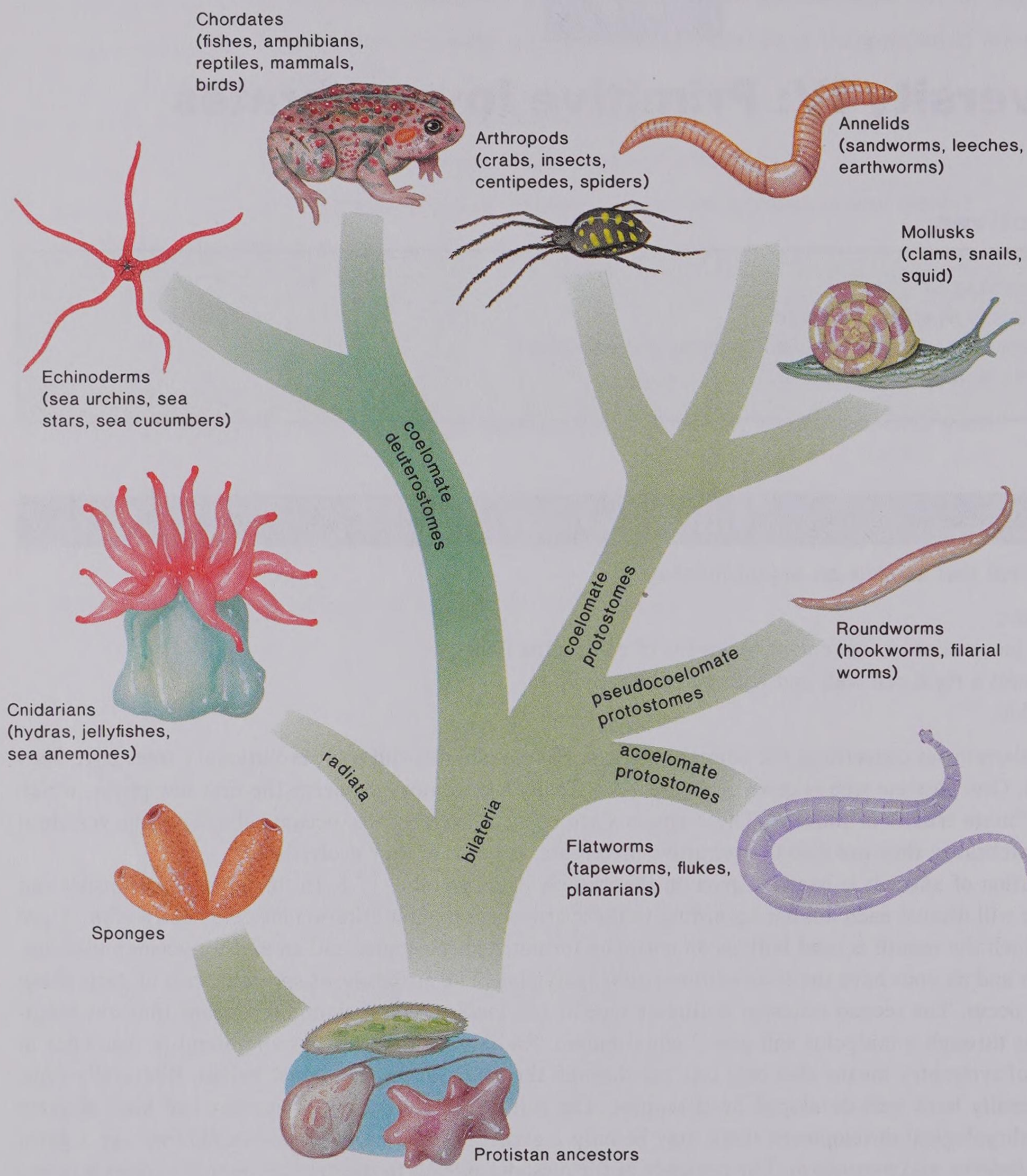


Table 17.1 Primitive* versus Advanced Animals

	Most Primitive	Primitive	Advanced	Most Advanced
Body plan	None	Sac plan	Tube-within-tube	Tube-within-tube, with specialization of parts
Symmetry	None	Radial	Bilateral	Bilateral with cephalization
Germ layers	None	2	3	3
Level of organization	None	Tissues	Organs	Organ systems
Coelom	Acoelomate	Acoelomate	Pseudocoelom	True coelom
Segmentation	Nonsegmented	Nonsegmented	Segmented	Segmented with specialization of parts

*Primitive means closer to the ancestral condition, not necessarily "simple."

Sponges (Phylum Porifera)

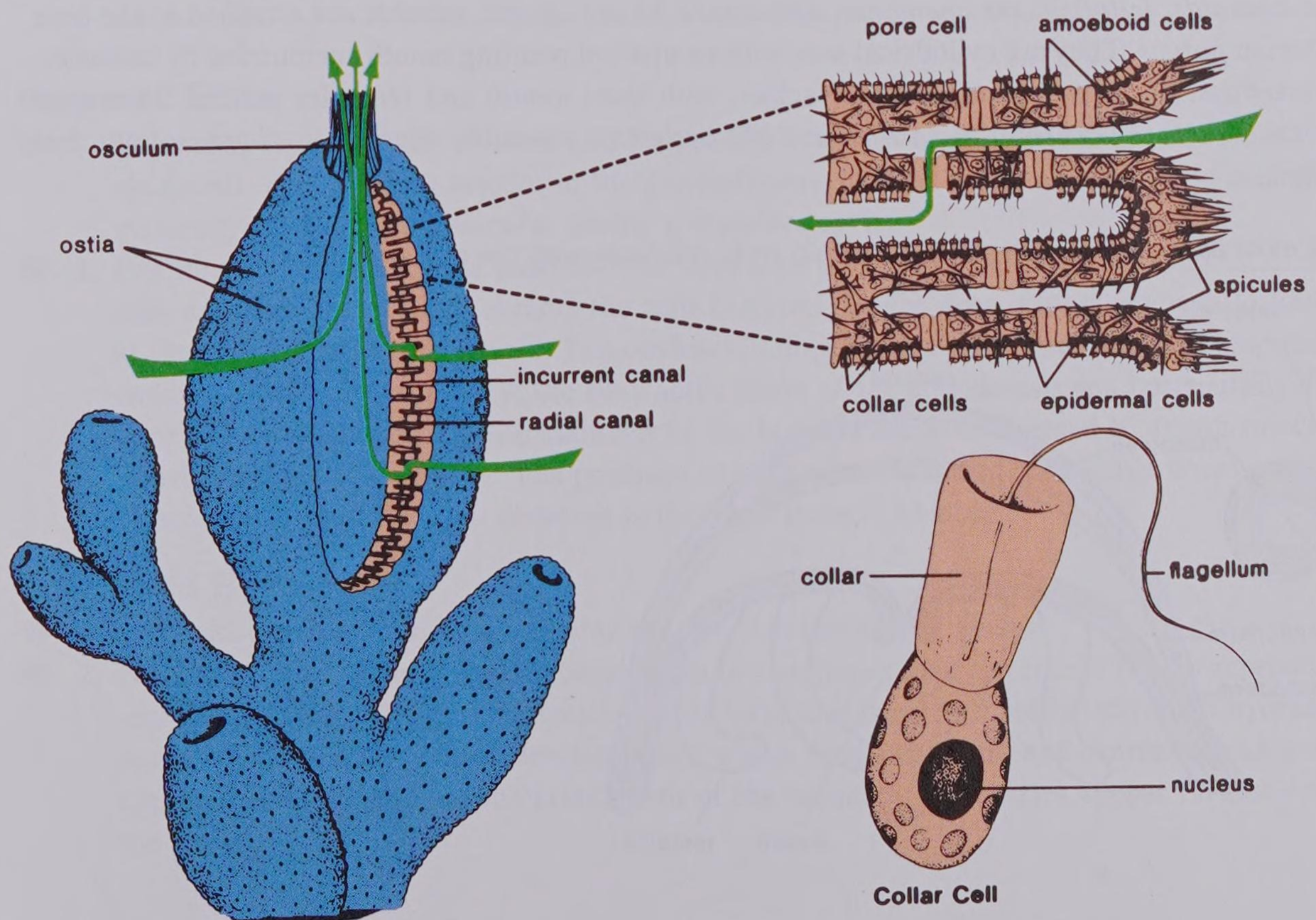
Sponges are multicellular animals not very different in many respects from certain of the protozoa. They live in water, mostly marine, attached to rocks, shells, and other solid objects. They are constructed in such a way that water, bearing food and oxygen, is caused to flow through their bodies, bringing in food particles and oxygen and removing waste and reproductive cells.

Grantia

Preserved Specimen

1. Examine a preserved sponge *Grantia* (fig. 17.2). Note the relatively large *osculum* (main excurrent opening) and the *ostia* (multiple incurrent pores).
2. Examine a specimen cut in half.

Figure 17.2 Sponge anatomy.



3. Note the central cavity and radial canals. Water enters the sponge through the *ostia* and leaves through the *osculum*. You may also be able to distinguish fine projections over the body and especially encircling the osculum. These are *spicules,* which are considered to be the skeleton of the sponge, serving to support and protect it from predation.

Prepared Slide

Examine a prepared slide of *Grantia* and find the *collar cells* that line the radial canals. A current of water that flows from the pores up and out the osculum is maintained by the beating of the flagella of these cells. Nutrients in the water are engulfed by the phagocytic action of the collar cells. The engulfed food is then digested in food vacuoles and passed to amoeboid cells for distribution to other cells.

Spicules

Examine a prepared slide of spicules and *sketch* what you see.

Spicules may be made of glass (silica), protein, or calcium carbonate. The type of spicule is used to classify sponges.

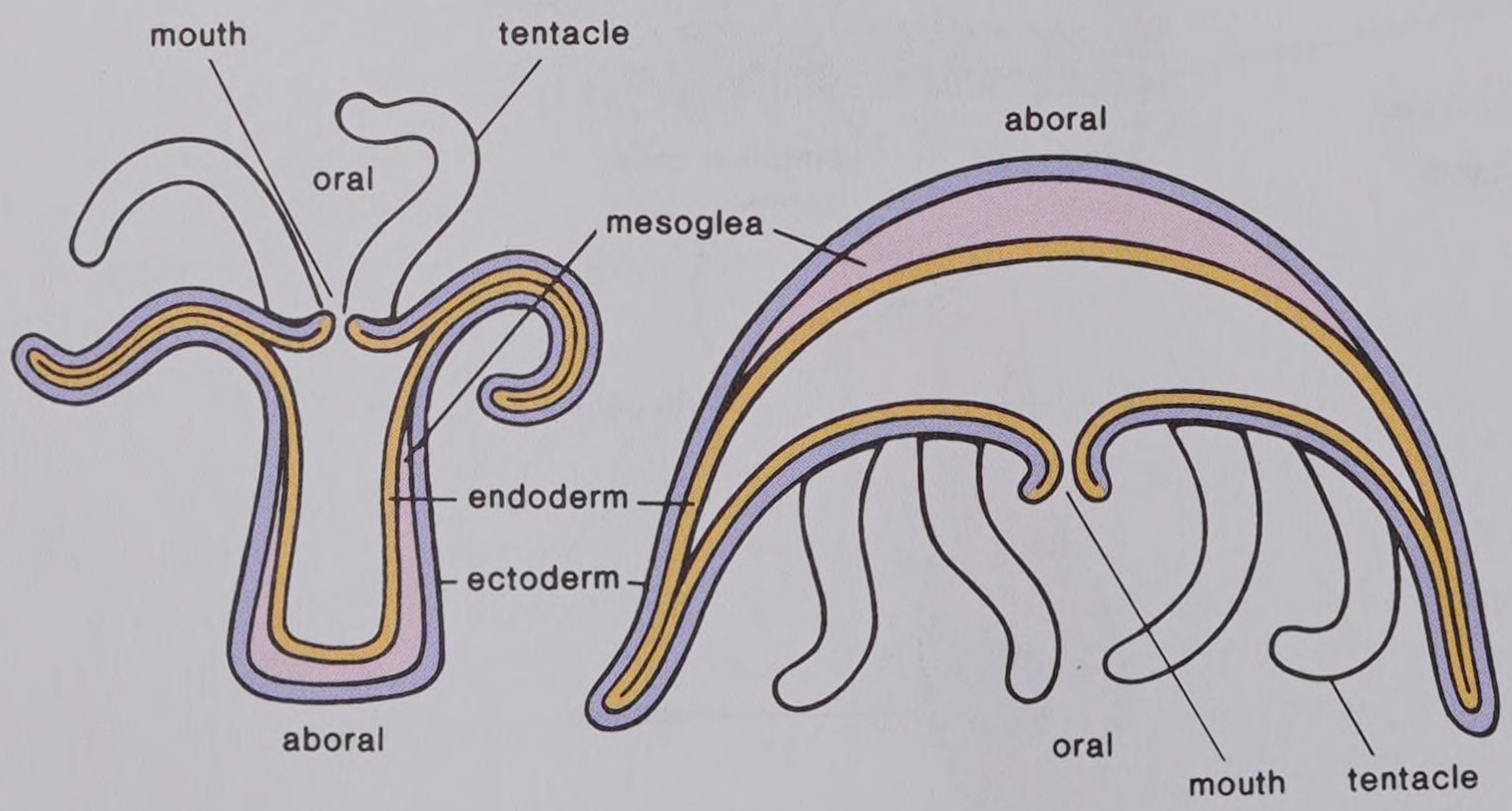
Discussion

Sponges have those features listed as most primitive in table 17.1. They are the only animal in which the single large opening is used only as an exit, and they have numerous small, incurrent openings (ostia). They are considered to be *asymmetrical* because no cut gives 2 equal halves. Their bodies are made up of a collection of cells rather than tissues. Also striking is the fact that sponges are *sessile:* that is, they remain in one place. The only evidence of locomotion is the beating of the flagella of the collar cells and the swimming zygote.

Cnidarians (Phylum Cnidaria)

This phylum includes the *hydroids, jellyfish, sea anemones,* and *corals.* Many aquatic animals are attached at the base (*sessile*), such as the cnidarian *polyps.* They are cylindrical sacs with an upward pointing mouth surrounded by tentacles. The swimming jellyfish (*medusae*) are less cylindrical, yet saclike, with their mouth and tentacles pointed downward (fig. 17.3). Other cnidarians alternate between the 2 forms in a life cycle that resemble alternation of generations, but both generations are diploid.

Figure 17.3 Cnidarians exist as ***a.*** *polyps* with the oral side up or ***b.*** *medusae* with the oral side down.



S Examine the preserved representatives of the phylum that are on display and complete table 17.2.

Table 17.2 Representative Cnidarians

Example	Form (polyp or medusa)
1.	
2.	
3.	
4.	
5.	

Hydra (Class Hydrozoa)

Live

S 1. Examine a specimen of live *Hydra* with a binocular dissecting microscope or hand lens (fig. 17.4). Note the following: *foot, tentacles,* and *mouth.*

S 2. Touch one of the tentacles very gently with a dissecting needle. What happens? _____

_____

S 3. Mount a living *Hydra* on a glass slide with a coverslip and examine a tentacle. Note the *stinging cells* that appear as swellings on the tentacles.

S 4. Tap the coverslip or add a drop of *vinegar* (5% acetic acid), if available, and note what happens to the stinging cells.

_____

Describe the structure and function of these *nematocysts* (stinging cells). _____

_____

Prepared Slide

S 1. Examine prepared slides of cross and longitudinal sections of *Hydra* (fig. 17.4*a*). Note the following: the *epidermis,* derived from ectoderm; the *gastrodermis,* derived from endoderm; the *mesoglea,* a packing material; and the *gastrovascular cavity,* a digestive cavity.

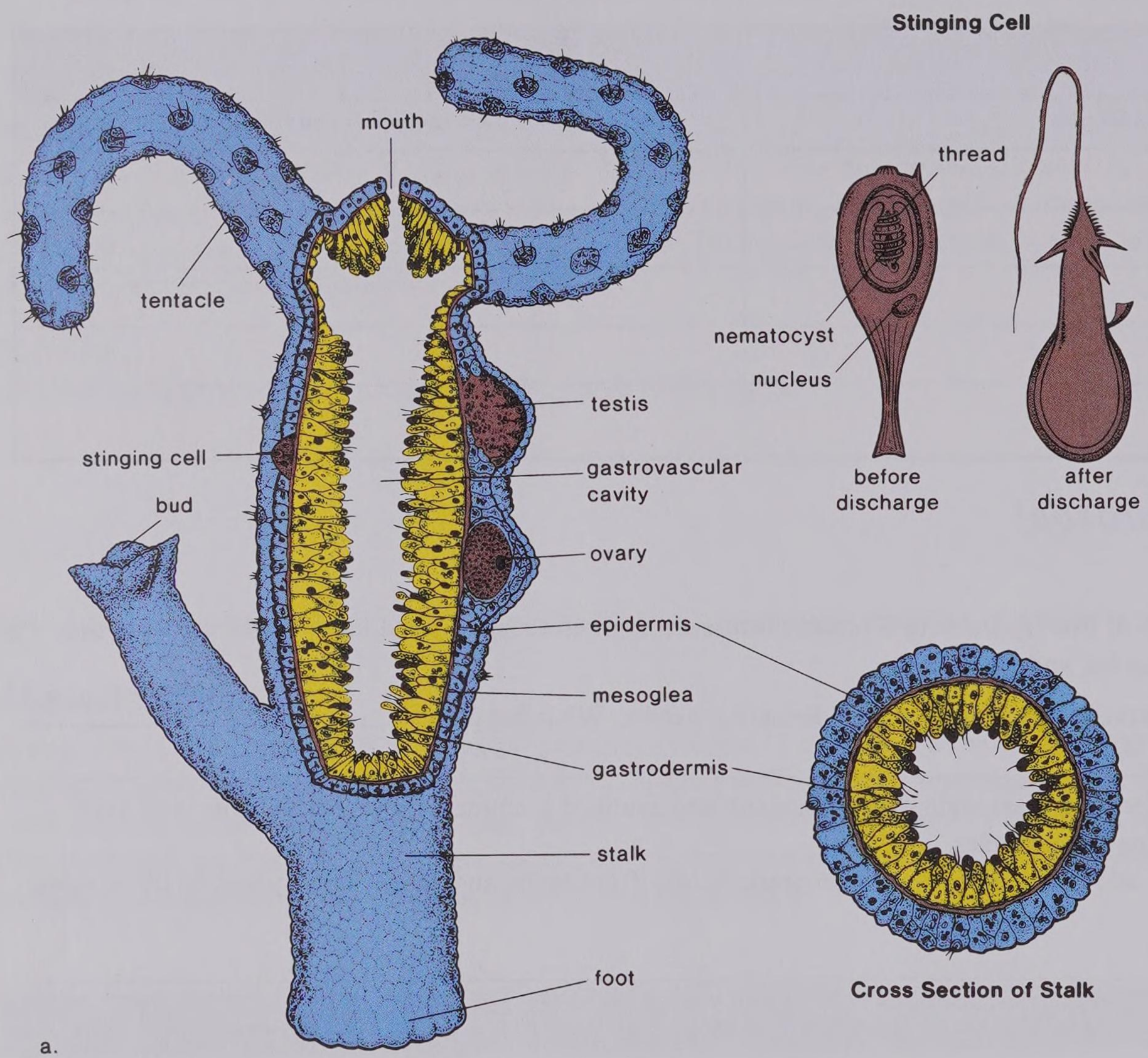
S 2. Examine the epidermis and gastrodermis with high power. Do you find any cells? _____ Although you may not have been able to observe the cells in detail, *Hydra* does possess *contractile fibers,* which are present in the cells of both tissue layers. The contractile fibers of the epidermal cells run longitudinally so that their contraction shortens the body; the contractile fibers of the endodermis run horizontally around the body so that their contraction decreases the diameter of the body. A *nerve net* formed by interconnecting nerve cells is located within the mesoglea. The presence of both nerve cells and contractile fibers enables the motor response to external stimuli that you observed earlier, but there is no brain.

Preserved Specimens

S 1. With the aid of a hand lens, examine preserved specimens of *Hydra.*

S 2. Note that some of these contain outgrowths or swellings along the trunk. *Hydra* reproduces both asexually and sexually. During asexual reproduction, buds form that develop directly into small hydras (fig. 17.4*b*). During sexual reproduction, Hydras develop testes, which produce sperm, and ovaries, which produce eggs. The testes are generally located near the attachment of the tentacles; the ovaries appear farther down on the trunk, near the foot.

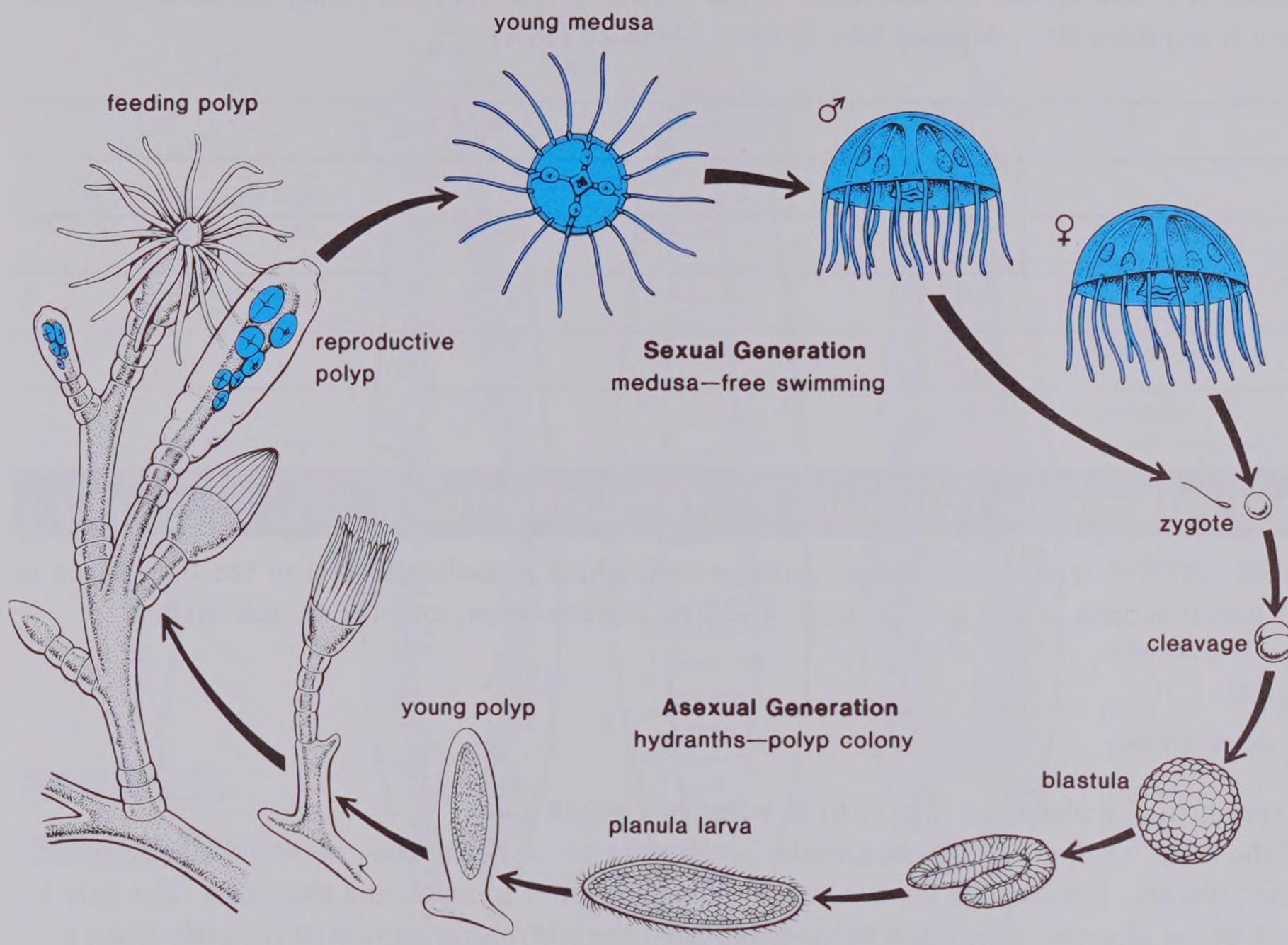
Figure 17.4 *Hydra.* ***a.*** Anatomy. Note stinging cells, which are shown in detail. ***b.*** Photomicrograph. Note bud on the left side.



a.

b.

Figure 17.5 Life cycle of *Obelia*.



Obelia (Class Hydrozoa)

Obelia is *polymorphic* (fig. 17.5). That is, the life cycle includes 2 distinct alternating phases: namely, the sessile, vegetatively reproducing *polyp* phase; and the motile, gametically reproducing *medusa* phase.

Polyp Colony

Ⓢ 1. Study a slide showing a polyp colony of *Obelia*. The colony grows by budding; fully grown buds usually remain attached to the colon.

Ⓢ 2. Note the bell-shaped *feeding polyps* with a mouth and a ring of stinging cell-bearing tentacles. The other polyps are *reproductive polyps*. In their interior, small, bell-shaped medusae develop that, when mature, escape from the polyp and assume a free-swimming existence as sexually active individuals. Considering the fact that the polyp stage of *Obelia* is sessile, what function is served by the free-swimming[1] medusa stage for the species?

_____

Medusa

Ⓢ 1. Examine a slide showing a medusa of *Obelia*.

Ⓢ 2. Note the tentacled bell and the central mouth. (Preparation of the slide unavoidably distorts the medusa—the umbrella is turned inside out.)

Discussion

Using *Hydra* as a representative cnidarian, it is possible to see that these animals have the characteristics listed under "primitive" (table 17.1).

[1]Many hydroid medusae stay attached, and dispersal occurs when motile larvae form after fertilization.

For each of the characteristics listed, explain what is *visible* (internal or external) in the structure of the *Hydra* that illustrates each characteristic, and explain its *usefulness* to the organism. Also indicate (using the letter abbreviations) if each characteristic is primitive (P), advanced (A), or most advanced (MA).

Sac plan _____

_____

_____

Radial symmetry _____

_____

_____

Flatworms (Phylum Platyhelminthes)

The flatworms are flattened dorsoventrally. They have an anterior end, which precedes the rest of the body when in motion; a posterior end; a dorsal surface, which is uppermost; a ventral surface below; and a right and left side.

Planaria (Dugesia)

Live

S 1. Examine a living specimen of a planarian (fig. 17.6) in water in a watch glass.
2. Note the shape of the body, head, eyespots, and auricles at the sides of the head. The *eyespots* are sensitive to light but do not form images. The *auricles* contain nerve endings sensitive to touch and chemicals. The mouth is located at the end of the pharynx, which can be extended from the midventral surface of the body when a planarian is feeding.
S 3. Place a small piece of meat such as hamburger in the watch glass and carefully watch the reaction of the worm.

Prepared Slides

S 1. Examine a whole mount of *Planaria* showing the branching digestive system. In what way is the digestive tract similar to the gastrovascular cavity of *Hydra*? _____

In what way is it different? _____

Would you expect an animal of this flatness to need a circulatory system? _____

Explain why or why not. _____

_____

S 2. Examine a cross section of a planarian under the microscope (fig. 17.7).
S 3. Locate the thin, compact *ectodermal* layer and the spongy *endodermal* cells lining the various branches of the digestive tract. Notice that the ectoderm and endoderm are separated by a third tissue layer, the *mesoderm,* consisting partly of muscle fibers that run in various directions. Flatworms and all the more advanced animals have 3 germ layers (table 17.1). Planarians have various *organs,* including a primitive pair of *nerve cords,* a tubular system of *excretory canals* terminating in "flame cells" that collect water and wastes, and a double set of *reproductive organs* (fig. 17.6).

Does your slide examination indicate that there is no space around the internal organs? _____

Does *Planaria* have a coelom? _____

Discussion

S Using *Dugesia* as an example, observe that free-living flatworms have some characteristics that are listed as primitive and some that are listed as advanced.

Figure 17.6 Anatomy of a planarian. ***a.*** Excretory system with flame cells shown in detail. ***b.*** Nervous system (note ladder appearance). ***c.*** Reproductive system and digestive system. ***d.*** Photomicrograph of numerous planarians.

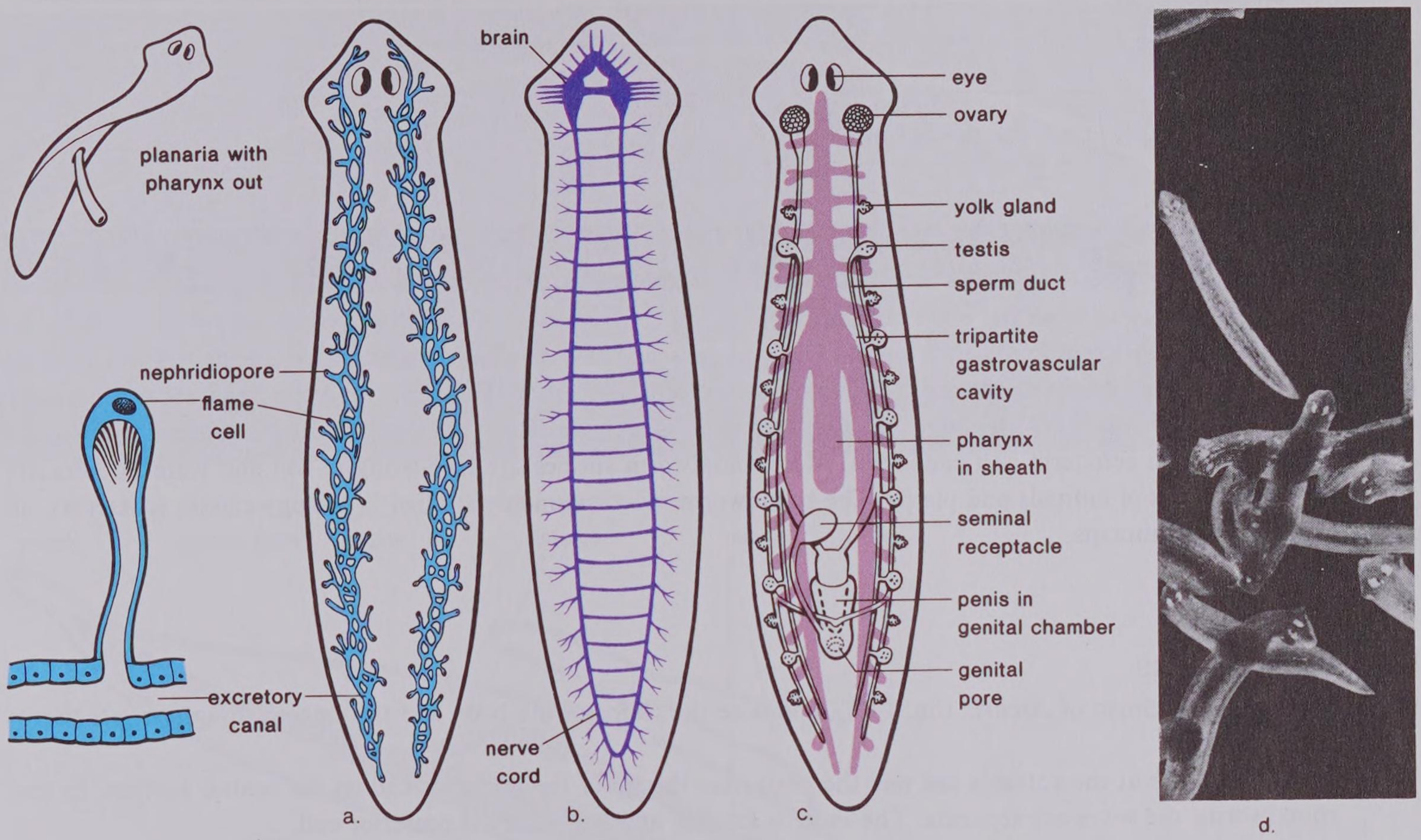
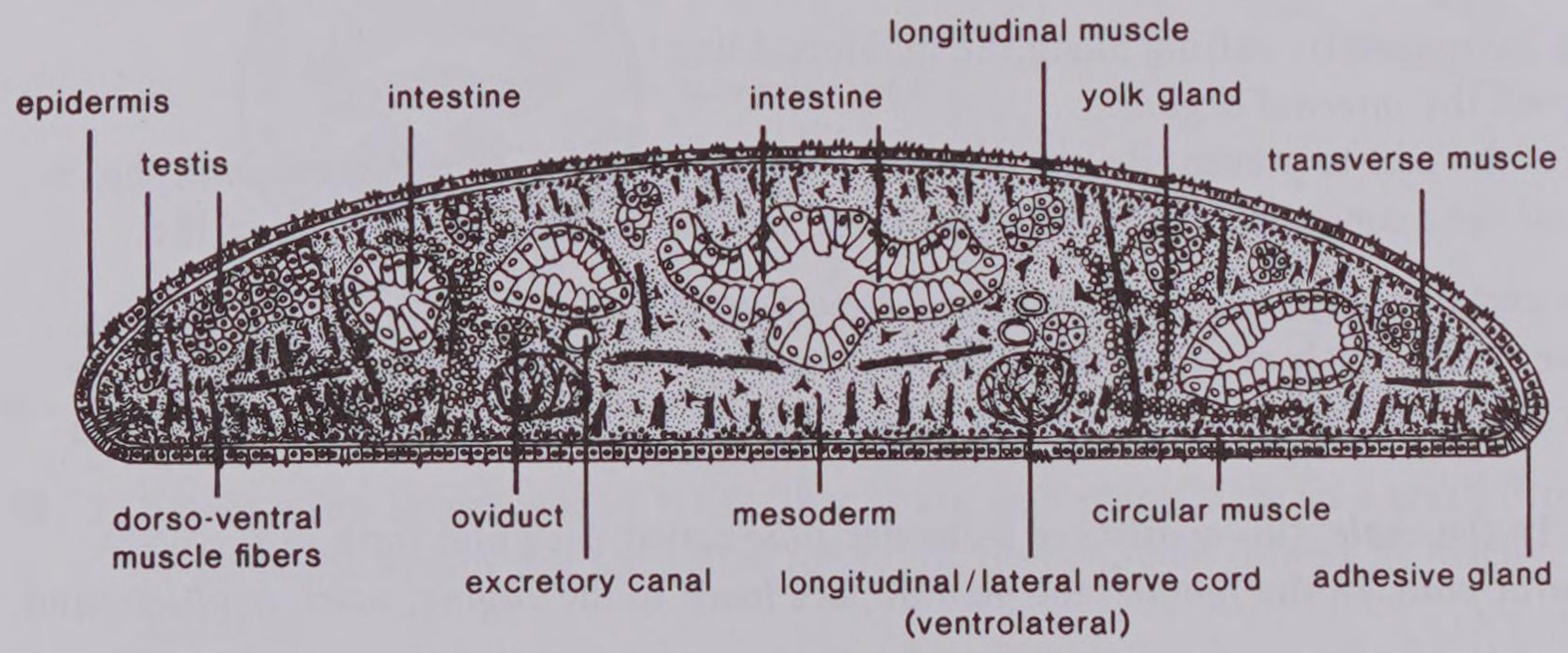


Figure 17.7 Cross section through anterior end of a planarian as it would appear under the microscope.



For each of the characteristics listed, explain what is *visible* (internal or external) in the structure of the *Dugesia* that illustrates each characteristic, and explain its *usefulness* to the organism. Also indicate (using the letter abbreviations) if each characteristic is primitive (P), advanced (A), or most advanced.

Sac plan _____

_____

_____

Bilateral symmetry _____

_____

_____

Organ level or organization _____

_____

Acoelomate _____

_____

How many germ layers are present in *Dugesia?* _____

Roundworms (Phylum Nematoda)

Roundworms have 2 features we have not encountered before, the *tube-within-a-tube* body plan and a *pseudocoelom.* The former characteristic represents an advance, since the presence of both a mouth and an anus leads to specialization of parts in higher phyla. A pseudocoelom, although incompletely lined with mesoderm (due to a separate evolutionary origin), allows space for the organs to bulge into and freedom of movement that is not possible when mesoderm completely fills the space between ectoderm and endoderm. Most roundworm species are free-living in soil and water; others are parasites in the tissues of animals and plants. The roundworm most commonly studied in biology classes is *Ascaris,* an intestinal parasite of humans.

Ascaris

Preserved Worms

- S 1. Examine a specimen of *Ascaris* (fig. 17.8), and note the *shape* of the body and the smooth, tough *cuticle* that covers it.
- S 2. Find the *mouth* at the anterior end and the *anus* near the tip of the posterior end on the ventral surface. In the roundworms the sexes are separate. The *male* is smaller and has a curved posterior end.

Dissection

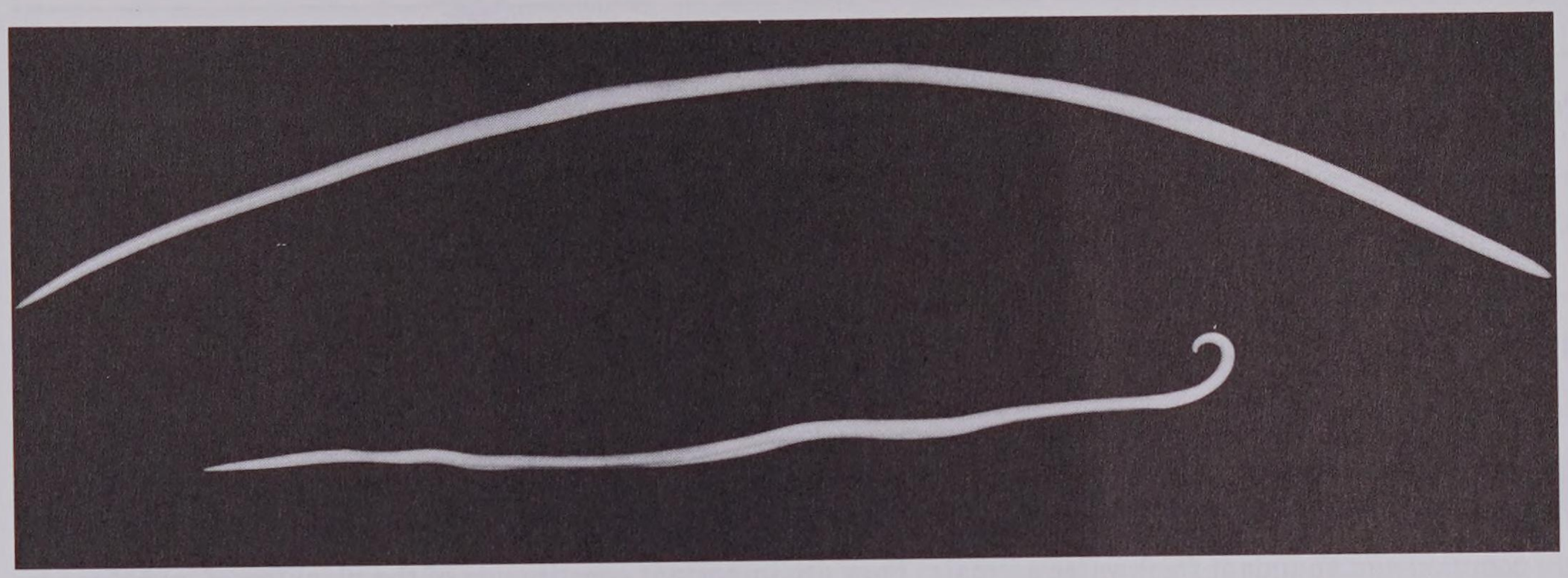
- S 1. Place a specimen in a dissecting pan, pinning the anterior and posterior ends to the wax with the dorsal surface exposed.
- S 2. Carefully slit open the worm *longitudinally,* cutting along the middorsal line.
- S 3. Now, pin open the body to expose the *internal organs.*
- S 4. Add a small amount of *water* to the pan to prevent drying out. Note the body cavity, or *pseudocoelom,* that is now evident. Note also the inner tube consisting of the digestive tract and the outer tube made up of the muscular body wall. Does this explain the phrase "tube-within-a-tube plan"? _____
- S 5. Examine the flattened *digestive tract,* which extends within the body cavity from the mouth to the anus. The anterior end forms a muscular *pharynx* used to suck food materials into the mouth. The rest of the tract is a tube that passes to the anus.
- S 6. Note the reproductive system. In the *male,* this consists of a slender *tube* coiled back and forth in the body cavity and attached at the *genital pore.* In the *female,* the genital pore leads to the *vagina, uteri, oviducts,* and *ovaries,* in the order named.
- S 7. Identify the organs and structures that are visible in the roundworm with the aid of figure 17.8.
- S 8. List the visible organs and structures: _____

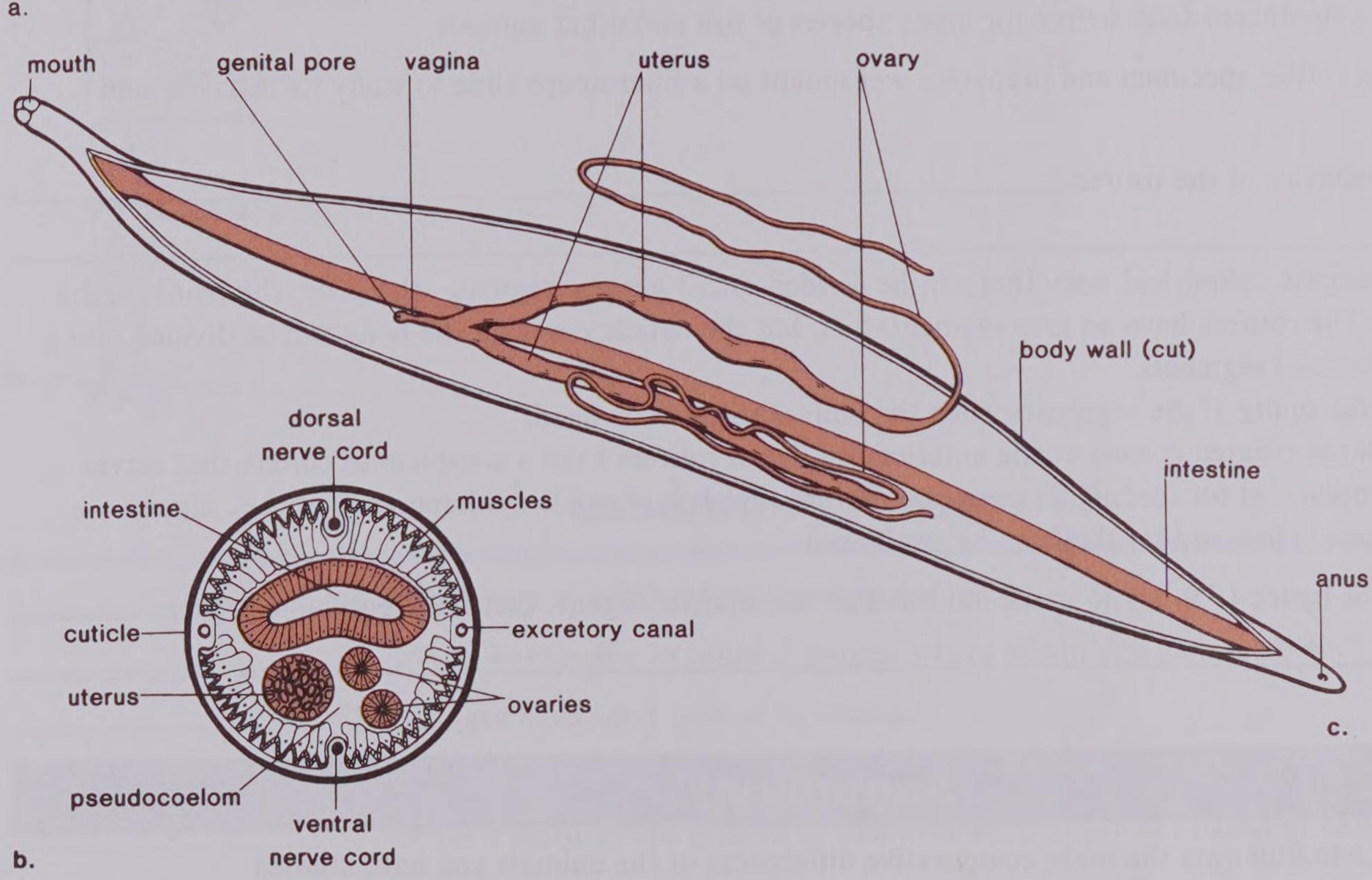
Vinegar Eels

Vinegar eels are tiny, free-living nematodes that can live in unpasteurized vinegar.

- S 1. Examine a sample from a culture of worms and observe their active, whiplike *swimming* movements. This thrashing sort of motion may be related to the fact that nematodes have longitudinal muscles only; they lack circular muscles.

Figure 17.8 *Ascaris.* ***a.*** Photograph of female (larger) and male (smaller). ***b.*** Cross section of female worm as it would appear under the microscope. ***c.*** Diagram of female internal anatomy following longitudinal dissection.





2. Select a few larger worms for further study, and place them in a small drop of *vinegar* on a clean microscope slide. If the worms are too active for study, they may be slowed by briefly warming them or by adding methyl cellulose.
3. Try to observe the tubular *digestive tract,* which begins with the mouth and ends with the anus. Also, it may be possible to make out some of the *reproductive organs,* particularly in a large female worm.

Discussion

If roundworms are discussed according to table 17.1, they have some primitive features and some advanced features. For each of the characteristics listed, explain what is *visible* (internal or external) in the structure of the roundworm that illustrates each characteristic, and explain its *usefulness* to the organism. Indicate (using the letter abbreviations) if each characteristic is primitive (P) or advanced (A) or most advanced (MA).

Tube-within-a-tube plan _____

_____

_____

Bilateral symmetry _____

_____

_____

Pseudocoelom _____

_____

Non segmented _____

_____

How many germ layers are present? _____

Rotifers (Phylum Rotifera)

Rotifers are common and abundant freshwater animals. They are important constituents of the plankton of lakes, ponds, and streams and are a significant food source for many species of fish and other animals.

S 1. Obtain a living rotifer specimen and prepare a wet mount on a microscope slide to study its behavior and appearance.

S 2. Describe the behavior of the rotifer. _____

_____

S 3. Observe the elongate cylindrical body that can be divided into 3 general regions—the *head,* the *trunk,* and a posterior *foot*. The rotifers have no true segmentation, but the cuticle covering the body can be divided into a number of superficial segments.

S 4. Observe the *telescoping* of the segments when the animal retracts its head.

S 5. Note also the large ciliated *corona* at the anterior end. Most rotifers have a conspicuous corona that serves both for locomotion and for feeding. It creates a current that brings smaller microorganism (i.e., algae, protozoa, bacteria) close so that they can be swallowed.

S 6. With the help of figure 17.9, try to make out some of the internal organs. List the identifiable organs: _____

_____

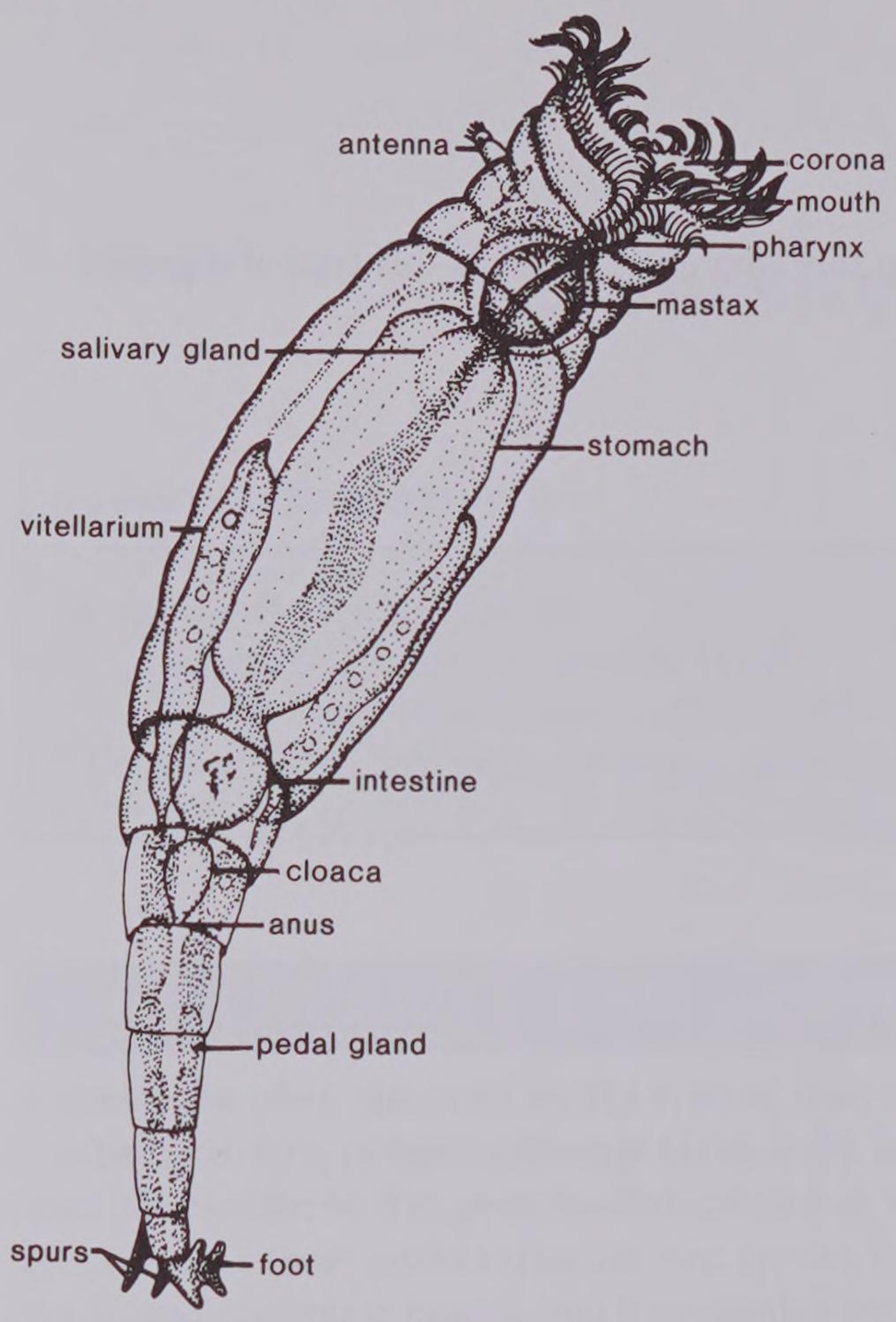
Summary

S Complete table 17.3 to illustrate the main comparative differences of the animals you have studied.

Table 17.3 Comparative Features

Name	Symmetry	No. of Germ Layers	Body Plan	Organs
Sponges				
Cnidarians				
Flatworms				
Roundworms				
Rotifers				

Figure 17.9 A rotifer, *Philodina.*



Laboratory Review 17

_____ 1. What type of symmetry is found in sponges?

_____ 2. If you were going to collect sponges, where would you look for them?

_____ 3. Sponges have what type of locomotion?

_____ 4. How is digestion accomplished in cnidarians?

_____ 5. What 2 features are present in roundworms that are not found in flatworms?

_____

_____ 6. Are the sexes separate in *Ascaris?*

7. Contrast the 2 stages found in *Obelia.*

8. Contrast the symmetry of a flatworm with that of a cnidarian.

9. What is a complete digestive tract? Name an organism studied in this laboratory that has this type of digestive system.

Reference

Wodsedalek, J. E., and Lytle, C. F. 1971. *General Zoology Laboratory Guide*. Dubuque, IA: Wm. C. Brown Publishers.

Laboratory

18

Diversity V: Protostomes

Learning Objectives

Students should be able to

1. identify and classify all animals studied;
2. identify the anatomical structures studied in the laboratory and state the function of each;
3. evaluate the animals according to table 17.1.

Introduction

Animals are often classified by the type of body cavity they have. The so-called primitive vertebrates are *acoelomate* (no body cavity), *pseudocoelomate* (false body cavity), or *coelomate* (have a true coelom), a body cavity completely lined by mesoderm. The *protostomes* are one of the 2 branches of the evolutionary tree of animals (fig. 17.1) that are coelomate. In these protostomes, the first (*protos*) embryonic opening becomes the mouth (*stoma*). In the deuterostomes, the second coelomate branch, the first opening becomes the anus and the second opening becomes the mouth. Today we are going to study the anatomy of 3 protostomes, the *clam,* the *earthworm,* and in particular, the *grasshopper.*

Mollusks (Phylum Mollusca)

The molluskan body is characterized by a ventral, muscular *foot* and a *visceral mass* enclosed in a thin tissue layer called the *mantle,* which usually secretes a calcareous shell. Modification of this basic organization has produced extreme variability among the mollusks. This phylum includes the *chitons, tooth shells, snails, slugs, clams, mussels, octopi,* and *squids.*

Preserved Clam (Venus)

External Anatomy

S 1. Examine the external *shell* (fig. 18.1). The more pointed end of the *valves* (the halves of the shell) is the posterior end, and the *umbo* curves toward the anterior end.
S 2. Find the *posterior* and *anterior* ends.
S 3. The valves are hinged together dorsally. Determine the *dorsal* and *ventral* regions of your clam.

Internal Anatomy

Valve

S 1. Carefully separate the *mantle* from one valve by inserting a scalpel into the slight opening of the valves.
S 2. Then place the hinge and umbo (blunt dorsal protrusion) down touching the dissecting pan.
S 3. Insert the scalpel between the mantle you just loosened and the valve.
S 4. The *adductor muscles* hold the valves together (see fig. 18.2 or 18.3). Cut the adductor muscle at the anterior and posterior ends by pressing the scalpel toward the pan.

Figure 18.1 External view of clamshell, left valve.

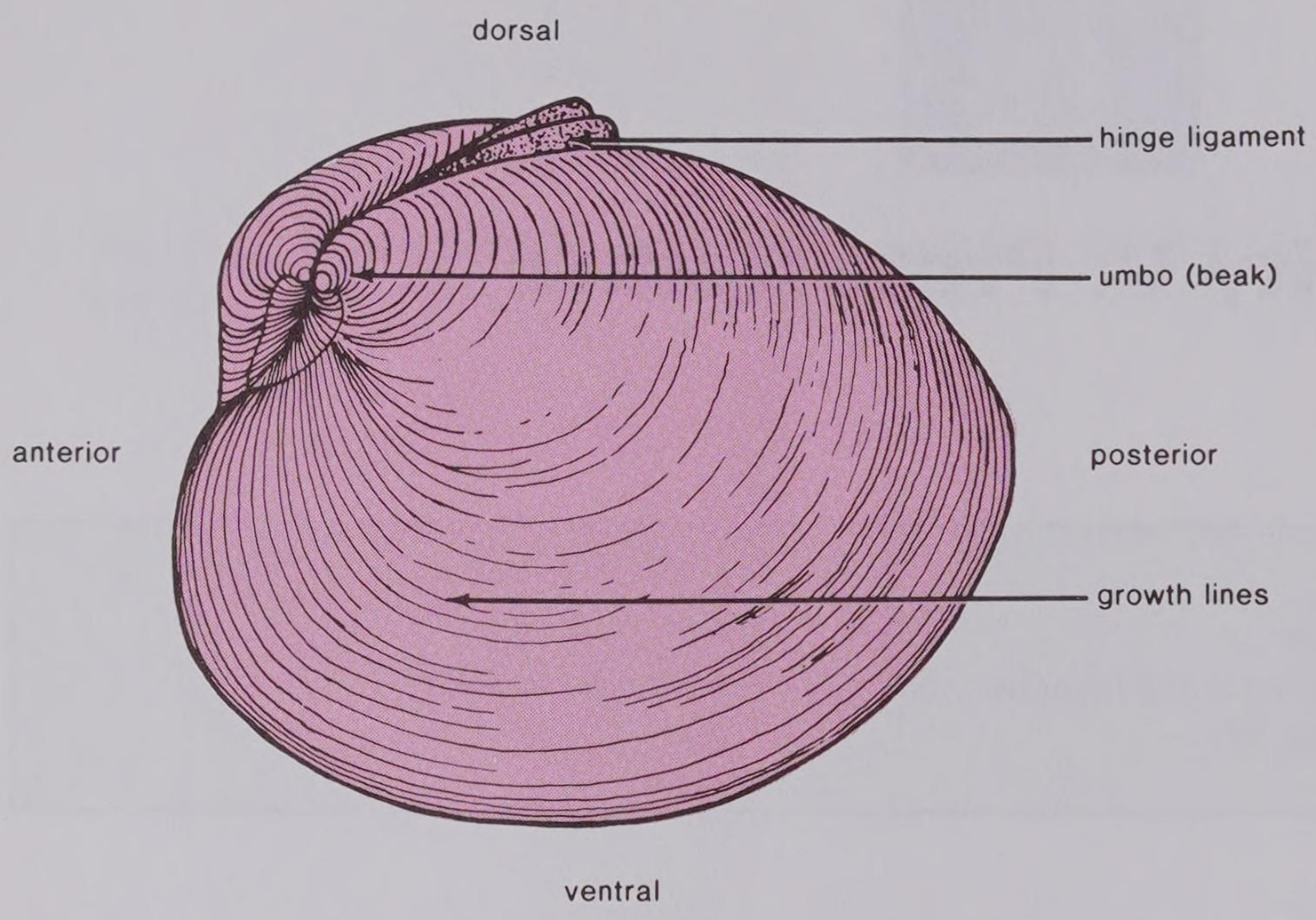


Figure 18.2 Clam shell. ***a.*** Inside shell showing muscle scars. ***b.*** Inside shell showing the mantle.

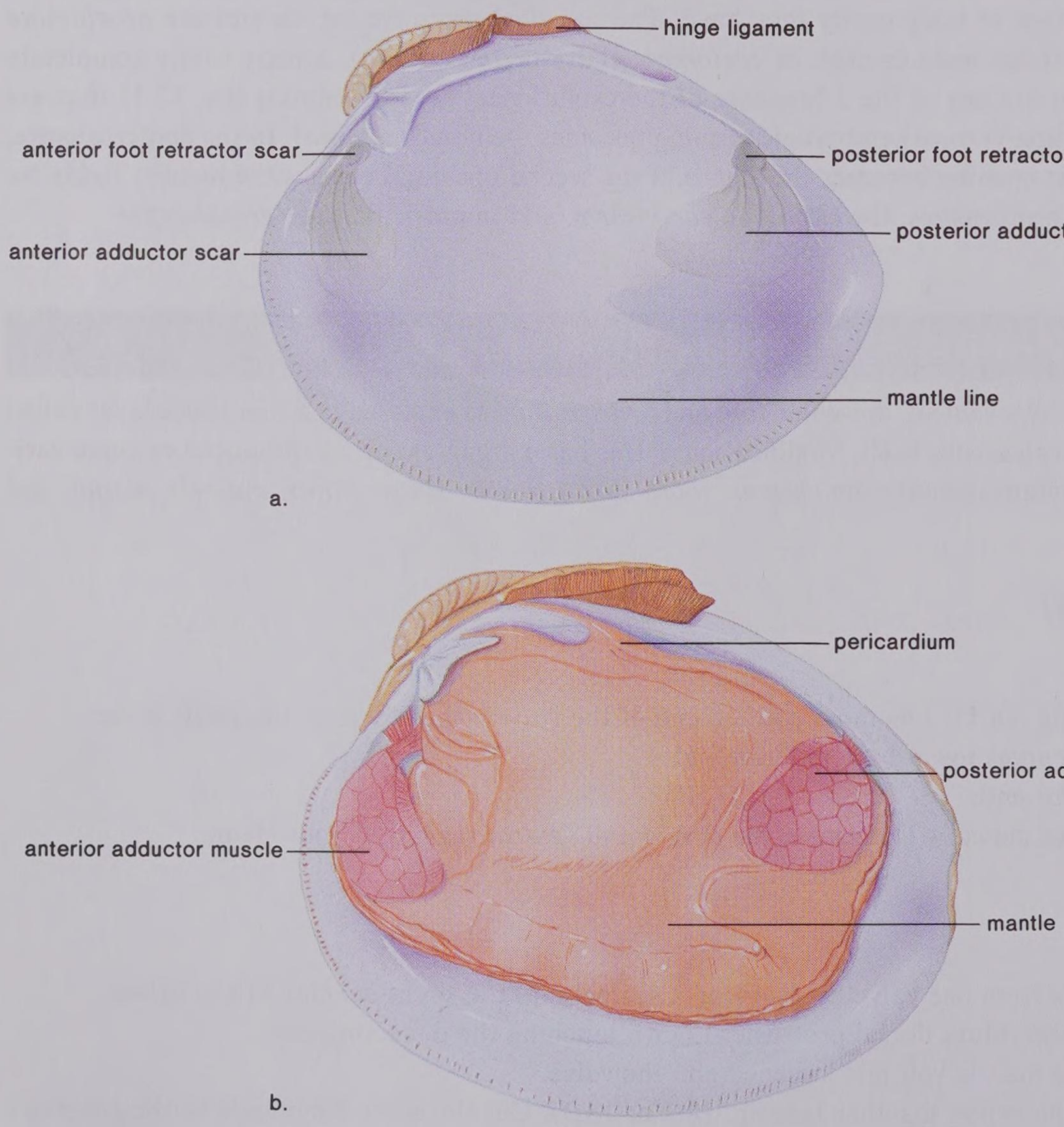
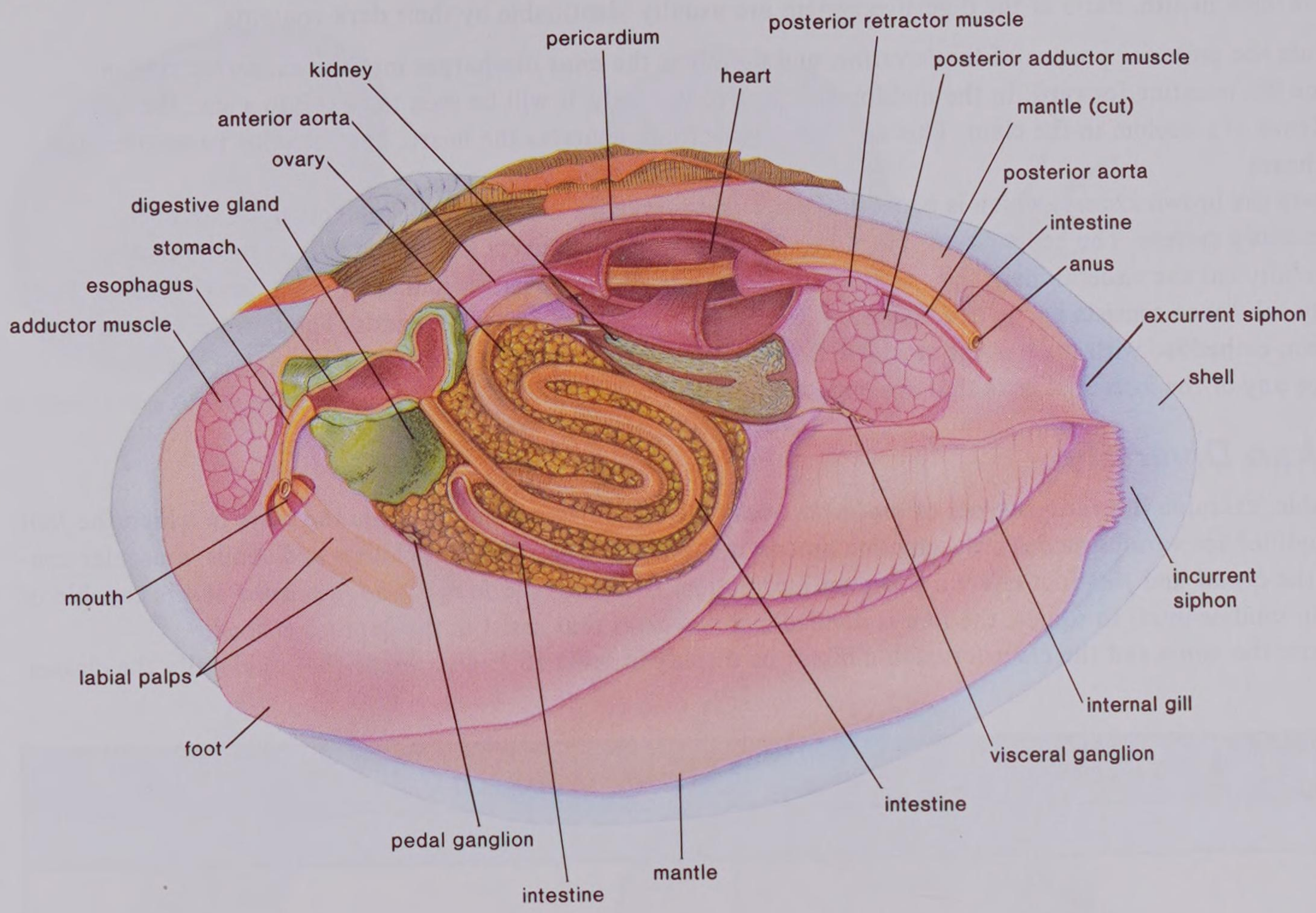


Figure 18.3 Longitudinal section of a clam.



5. After these muscles are cut, the valve may be lifted away carefully.
6. Examine the valve you removed (fig. 18.2*a*). Note the *concentric lines* of growth on the outside; the *hinge teeth* that interlock with the other valve; the remains of the *adductor muscles;* and the *mantle line*. The edge of the mantle secretes the shell. The inner layer of the shell is mother of pearl.
7. Examine the rest of the clam (fig. 18.2*b*) attached to the other valve. Notice the adductor muscles and the mantle, which lies over the visceral mass and foot.

Mantle Cavity (fig. 18.3)

1. Bring the 2 halves of the mantle together and notice that then you will see the *incurrent* (more ventral) and *excurrent siphons* at the posterior end. A stream of water comes in the incurrent siphon and goes out the excurrent siphon, driven by the beating of the cilia on the gills, and a stream of water passes through the porous gills in the mantle cavity.
2. Cut away the free-hanging portion of the mantle to expose the *mantle cavity* and *gills*.

The gills serve what function? The specialized cilia on the gills entrap food particles brought into the mantle cavity by way of the incurrent siphon, and the action of different cilia on the gill surfaces conveys them to the mouth.

Why is the clam called a suspension feeder? _____

Foot and Visceral Mass (fig. 18.3)

1. Identify the *foot* and *visceral mass* structures described following using figure 18.3 and your clam.

The main fleshy body of the clam is made up of 2 major parts that merge into each other: the tough, muscular foot for locomotion and, dorsal to the foot, the visceral mass, which is softer and rather plump. It contains the digestive and reproductive organs.

Toward the anterior and dorsal margin of the body are a pair of *labial palps*. These limp flaps of tissue channel food into the open mouth. Parts of the digestive system are usually identifiable by their dark contents.

2. Locate the posterior portion of the *intestine,* and note that the *anus* discharges into the *excurrent siphon.*
3. Trace the intestine forward. In the middorsal region of the body, it will be seen to pass into a sac, the only evidence of a coelom in the clam. This sac, the *pericardium,* contains the heart. The intestine passes through the heart.
4. Locate the brown *kidney,* which is beneath the pericardium. Like most mollusks, the clam has an open circulatory system. The heart pumps blood into the aorta, which delivers it to blood sinuses in the tissues.
5. Carefully cut the visceral mass and the foot into exact left and right halves and examine the cut surfaces. The pasty substance consists partly of reproductive organs and partly of digestive glands. The latter are greenish brown; embedded with them is the stomach. The intestine winds about in the visceral mass.
6. Trace any of the intestinal coils that are exposed.

Molluskan Diversity

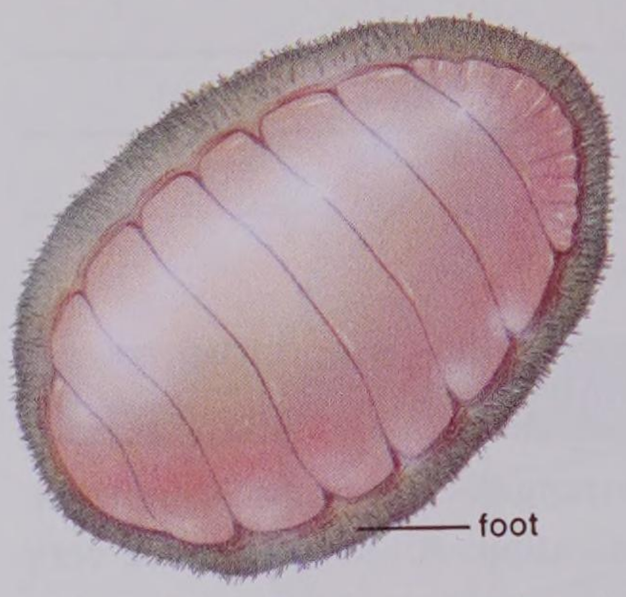
If available, examine the various types of *mollusks* your instructor has on display. Note the ways in which the *foot* has been modified for various modes of locomotion among the mollusks (fig. 18.4). In chitons and snails, muscular contraction in the *broad* and *flat* foot effect a creeping locomotion. In clams, the foot is *wedge-shaped* and is capable of burrowing in sand or mud. In squids, the foot is divided into *tentacles* that assist in the capture of food.

Write the *name* and the *class* for each mollusk on display in table 18.1. Use figure 18.4 to identify the classes.

Table 18.1 Examples of Mollusks

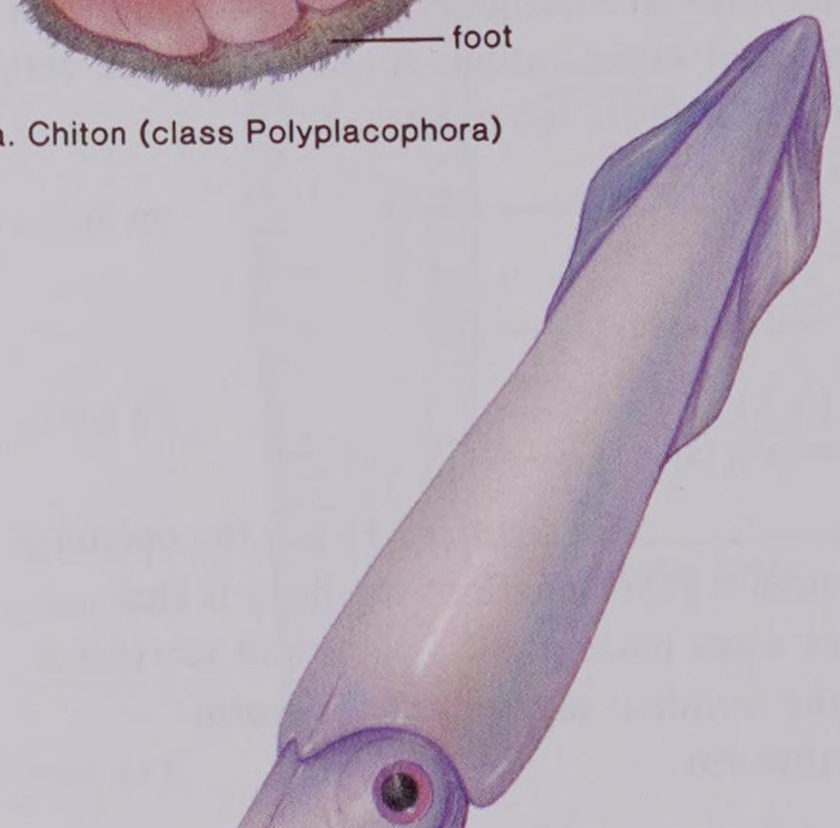
Name	Class

Figure 18.4 Classes of mollusks.

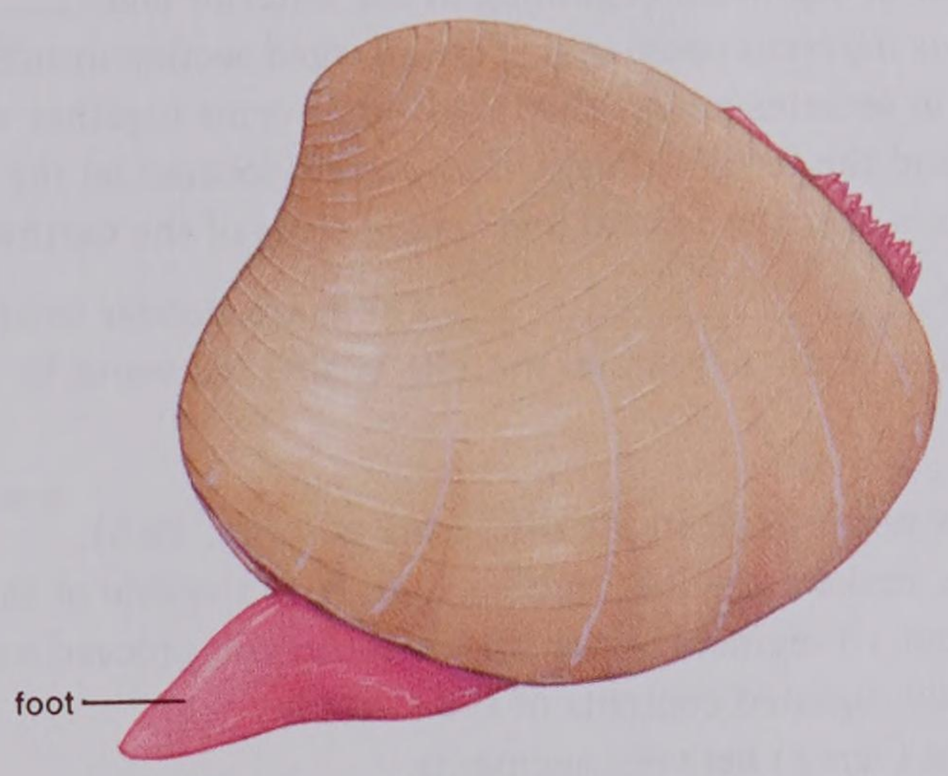


a. Chiton (class Polyplacophora)

b. Snail (class Gastropoda)



c. Squid (class Cephalopoda)



d. Clam (class Bivalvia)

Summary

For each of the characteristics listed, explain what is *visible* (internal or external) in the structure of the mollusks that illustrates each characteristic, and explain its *usefulness* to the organism. Also indicate (using the letter abbreviations) if each characteristic is primitive (P), advanced (A), or most advanced (MA). Refer to table 17.1 if necessary.

Tube-within-a-tube with specialization of parts _____

Bilateral symmetry with cephalization, such as in the squid and octopus _____

Organ systems _____

_____

Nonsegmented _____

_____

Annelids (Phylum Annelida)

Annelids are characterized by numerous *ringlike segments* (or somites). The internal arrangement of muscles, nerves, blood vessels, excretory ducts, and reproductive organs also exhibits a segmental organization. All segments are very similar except for the first 3, which form the *head,* and the last segment, which includes the *anus*.

Earthworm (Lumbricus)

External Anatomy

S 1. Note that the animal is cylindrical and has bilateral symmetry (fig. 18.5).

S 2. Locate the small projection that sticks out over the mouth.

S 3. Count the total number of segments beginning at the anterior end. _____ On segment 15 are the openings of the *sperm ducts* (*vas deferens* openings). The enlarged section around a short length of the body is the *clitellum.* The clitellum secretes mucus that holds the worms together when mating takes place and secretes a protective cocoon around the fertilized eggs. The *anus* is located on the terminal segment of the worm.

S 4. Lightly pass your fingers over the ventral and lateral sides of the earthworm.

Do you feel the *setae?* _____ These are slender bristles inserted into the dirt during locomotion. Both circular and longitudinal muscles enable the worm to locomote and change shape.

Internal Anatomy

S 1. Place the animal on its *ventral* side in the dissecting pan (fig. 18.6).

S 2. With your razor blade, make a shallow incision slightly to the side of the blackish *median dorsal* blood vessel.

S 3. Start your incision about 10 segments after the clitellum and proceed anteriorly to the mouth. If you see black ooze, this is the partially digested contents of the intestine.

S 4. Note the thin partitions (*septa*) between segments.

S 5. Lay out the body wall and *pin* every tenth segment to the wax of your pan.

S 6. Add water to prevent drying out.

The earthworm is *simultaneously hermaphroditic* (having both male and female sex organs functional at the same time). (Note: many animals change sex as they age and are called sequentially hermaphroditic.)

S 7. Locate the *seminal vesicles,* which are light-colored bodies in the ninth through twelfth segments. These vesicles house maturing *sperm* that have been formed in 2 pairs of testes within them.

S 8. Find the *sperm ducts* that pass to openings in segment 15. Four small, white spherical bodies called *seminal receptacles* lie in the ninth and tenth segments. These receive sperm from the partner during mating. The *ovaries* are in segment 13 but are too small to be seen.

S 9. Locate each of the internal structures described following.

The digestive tract begins at the *mouth,* which extends through the first 3 body segments. It opens into the swollen, muscular, thick-walled *pharynx,* which extends from segments 3 to 6. The *esophagus* passes from the pharynx through the fourteenth segment to the *crop.* Next is the *gizzard,* which lies in segments 17 and 18. The intestine extends from the gizzard to the *anus*.

The circulatory system is *closed:* the blood is always contained within vessels. The *dorsal blood vessel* is readily seen along the dorsal side of the digestive tract. A series of contractile, or muscular, *hearts* encircle the esophagus between segments 7 and 11, connecting the dorsal blood vessel with the ventral blood vessel.

The earthworm has a well-developed nervous system. The 2-lobed *brain* is located on the dorsal surface of the pharynx in the third segment. Two *nerves,* one on each side of the pharynx, connect the brain to a ganglion that lies below the pharynx in segment 4. The *ventral nerve cord* then extends along the floor of the body cavity to the last segment.

Figure 18.5 Earthworm, external view.

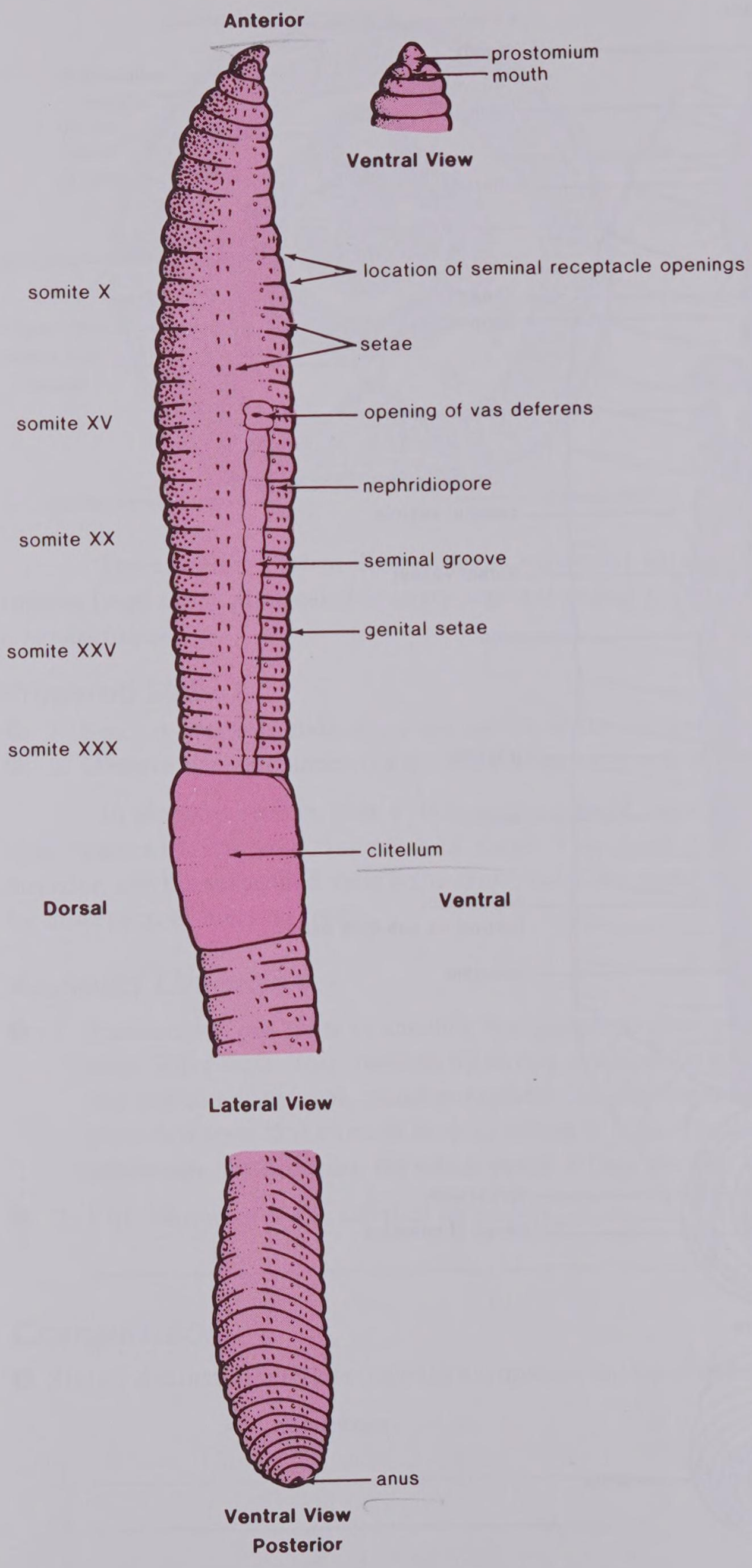


Figure 18.6 Earthworm anatomy, following dissection.

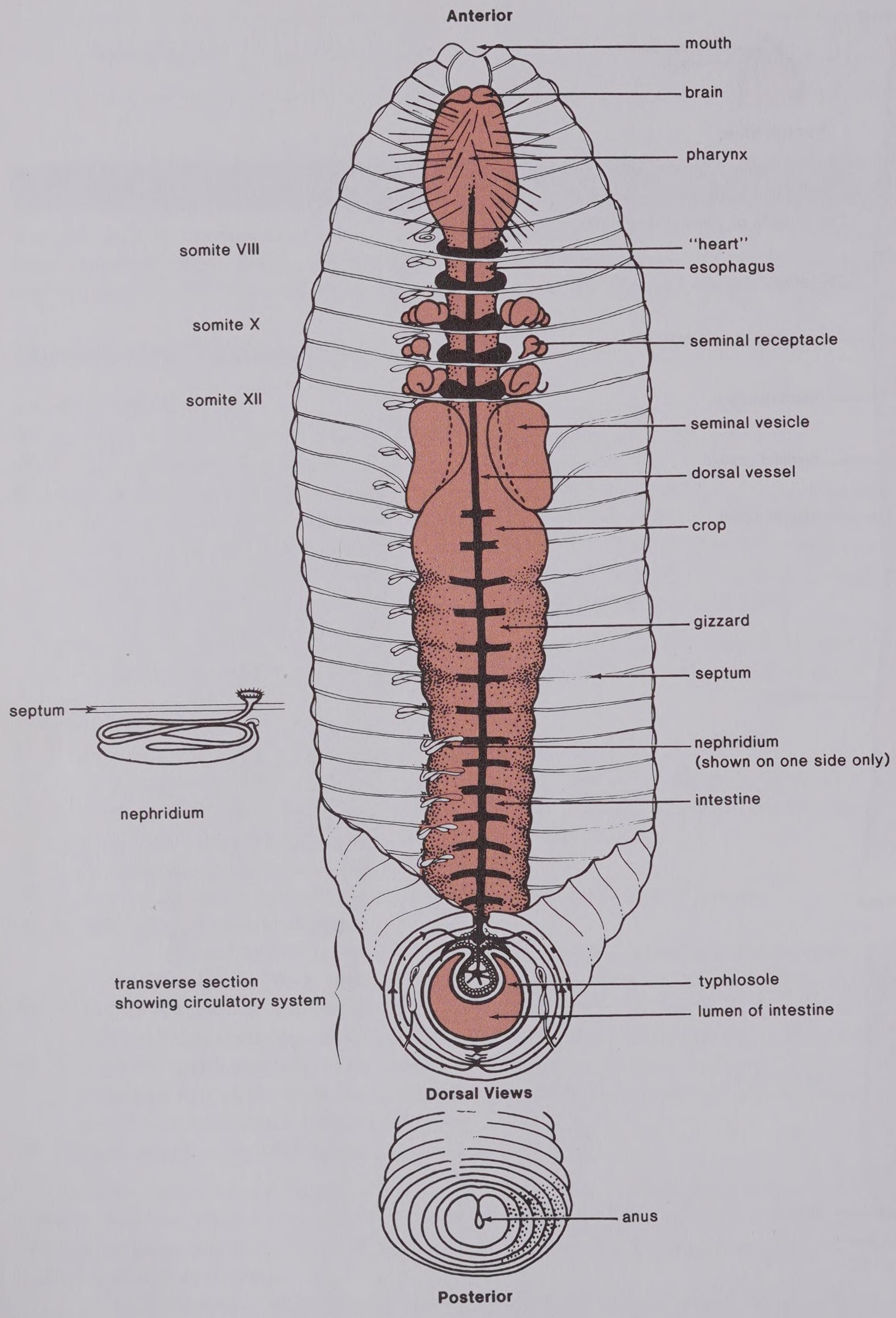
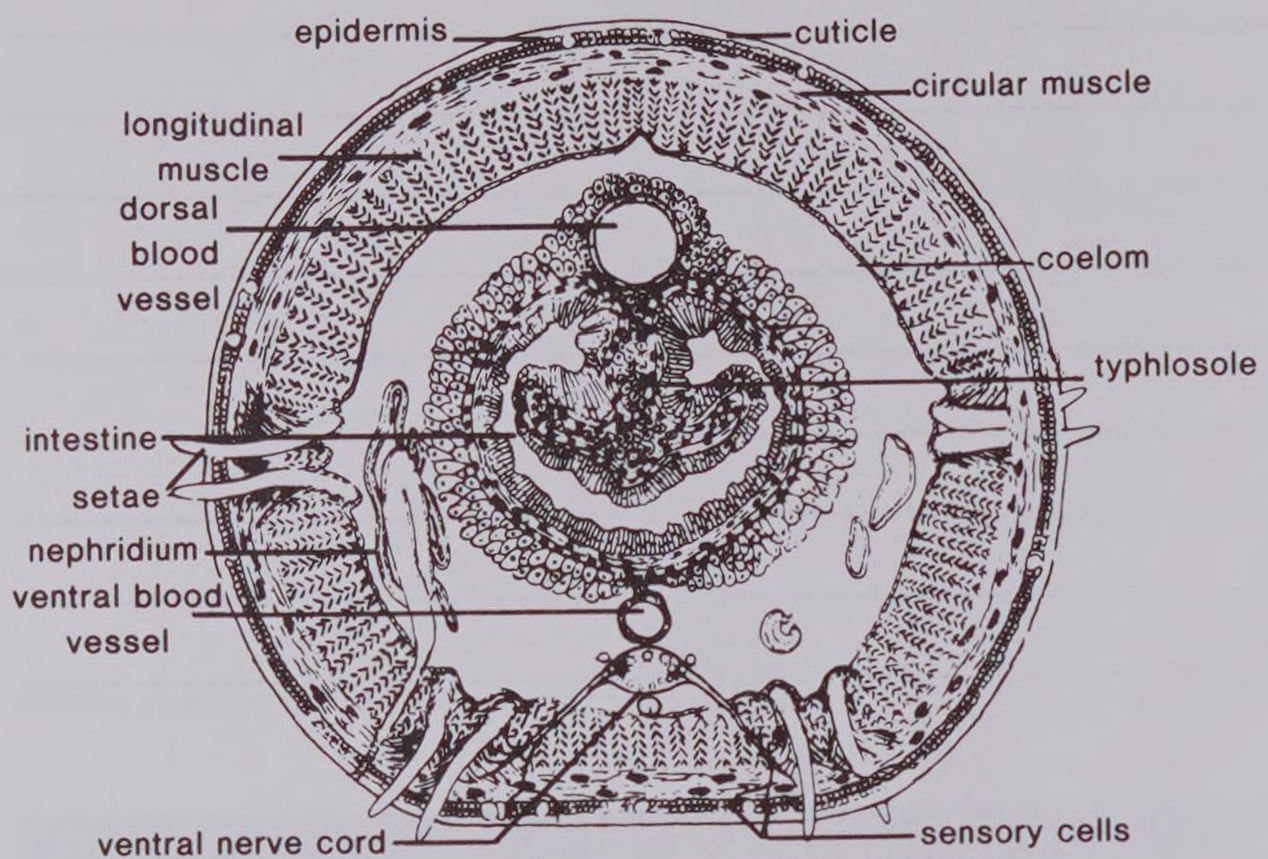


Figure 18.7 Earthworm, cross section as it would appear under the microscope.



The excretory system illustrates the segmental nature of earthworm anatomy. A pair of minute, coiled white tubules (*nephridia*) are located in every segment except the first 3 and the last. Each opens to the outside by means of a *nephridiopore*.

Prepared Slide

Ⓢ 1. Study a prepared slide of a cross section of the earthworm (fig. 18.7).

Ⓢ 2. Observe the slide under the binocular dissecting microscope and the compound light microscope.

In the cross section, note a thick outer circle of tissue, the *body wall*. Inward from the body wall is a relatively clear space with scattered fragments of tissue. This space represents the *coelom*. The inner circle is the wall of the *intestine,* and the suspended mass is the *typhlosole*. The typhlosole increases the surface area and thus increases the area for absorption of digested food.

Annelid Diversity

Ⓢ 1. Examine various types of annelids that your instructor may have on display. Note that the *polychaetes'* many setae differ from other annelids by having a well-differentiated anterior region with specialized sense organs, and paired appendages, called *parapodia,* on most segments. Some polychaetes are active *predators,* and others are *tubeworms* that capture food by means of cilia. The *leeches,* having an anterior and posterior *sucker,* are sometimes bloodsuckers, for which practice they are well known.

Ⓢ 2. List the names of the annelids on display. _____

_____

Comparison

Ⓢ State 3 distinct differences between earthworm anatomy and clam anatomy.

	Earthworm	Clam
1.	_____	_____
2.	_____	_____
3.	_____	_____

For each of the characteristics listed, explain what is *visible* (internal or external) in the structure of the annelids that illustrates each characteristic, and explain its *usefulness* to the organism. Also indicate (using the letter abbreviations) if each characteristic is primitive (P), advanced (A), or most advanced (MA). Refer to table 17.1 if necessary.

Tube-within-a-tube with specialization of parts _____

_____

_____

Bilateral symmetry with cephalization _____

_____

_____

True coelom _____

_____

Segmented _____

_____

Arthropods (Phylum Arthropoda)

About three-quarters of all described animal species are arthropods. In arthropods, the body is covered by a *chitinous exoskeleton* to which the muscles are attached internally. The body is segmented, and there is usually a distinct *head, thorax,* and *abdomen.* (The head and thorax may be fused into a single unit, the cephalothorax, and covered by a single sheet, the carapace.) *Joint appendages* and *simple* and/or *compound eyes* are present.

Crayfish (Class Crustacea)

External Anatomy and Behavior

S 1. Obtain a living crayfish (*Cambarus*), being sure to *pick it up firmly by the thorax.* Place the animal in a shallow pan or bowl of *water.* With the help of figure 18.8, locate the *head, thorax,* and *abdomen.*

S 2. Find the *carapace,* which covers the head and thorax. Together the head and thorax are called the *cephalothorax.*

S 3. Find the *rostrum, eyes, gill chambers, mouth,* and *anus.*

S 4. Locate the following appendages on one side of the body:

Appendages of Crayfish

Head

First antenna—equilibrium (contains a statocyst), touch and taste
Second antenna—touch and taste
Mandible—biting food
Maxillae (first and second)—food handling

Thorax

Maxillipeds (first, second, and third)—touch, taste, food handling
Walking legs (first through fifth)
first—pincer for offense and defense
second through fifth—walking

Abdomen

Swimmerets (first through fifth)—water circulation

Males
first and second—modified for copulation and transfer sperm from male to female

Females
Carry eggs and young

Uropod—swimming

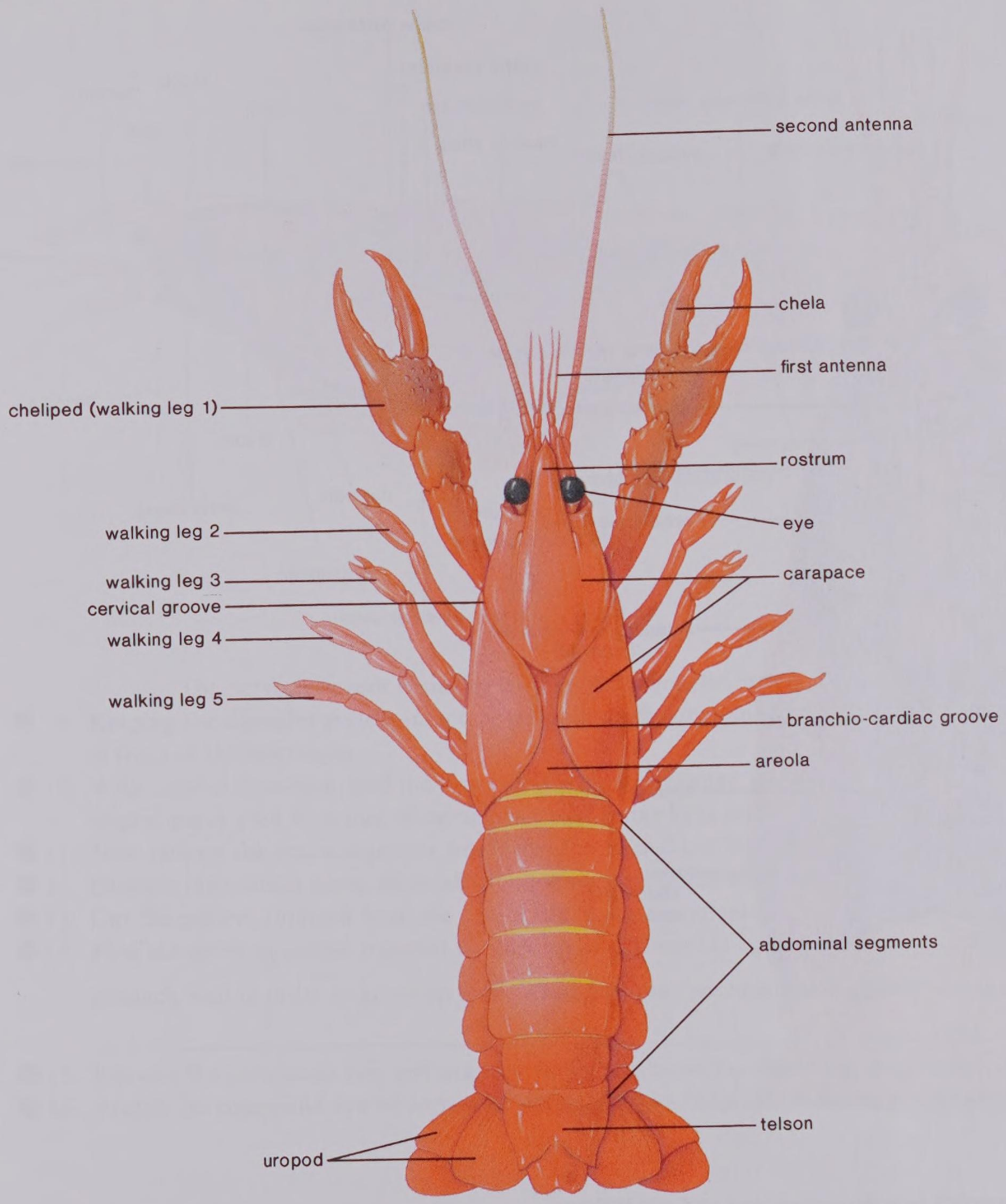
Are all the appendages jointed? _____

S 5. After placing the crayfish in a shallow pan of *water,* offer it some food (a small piece of earthworm, or fish).

S 6. Observe the method of feeding.

S 7. Observe the swimming and walking movements.
Which appendages are involved in feeding? _____ in swimming? _____
in walking? _____ How are the antennae used? _____

Figure 18.8 Crayfish, general dorsal appearance.



8. Turn your animal upside down.

   How does it right itself? _____

   _____

   How do you think the crayfish digs or burrows in the earth? _____

   _____

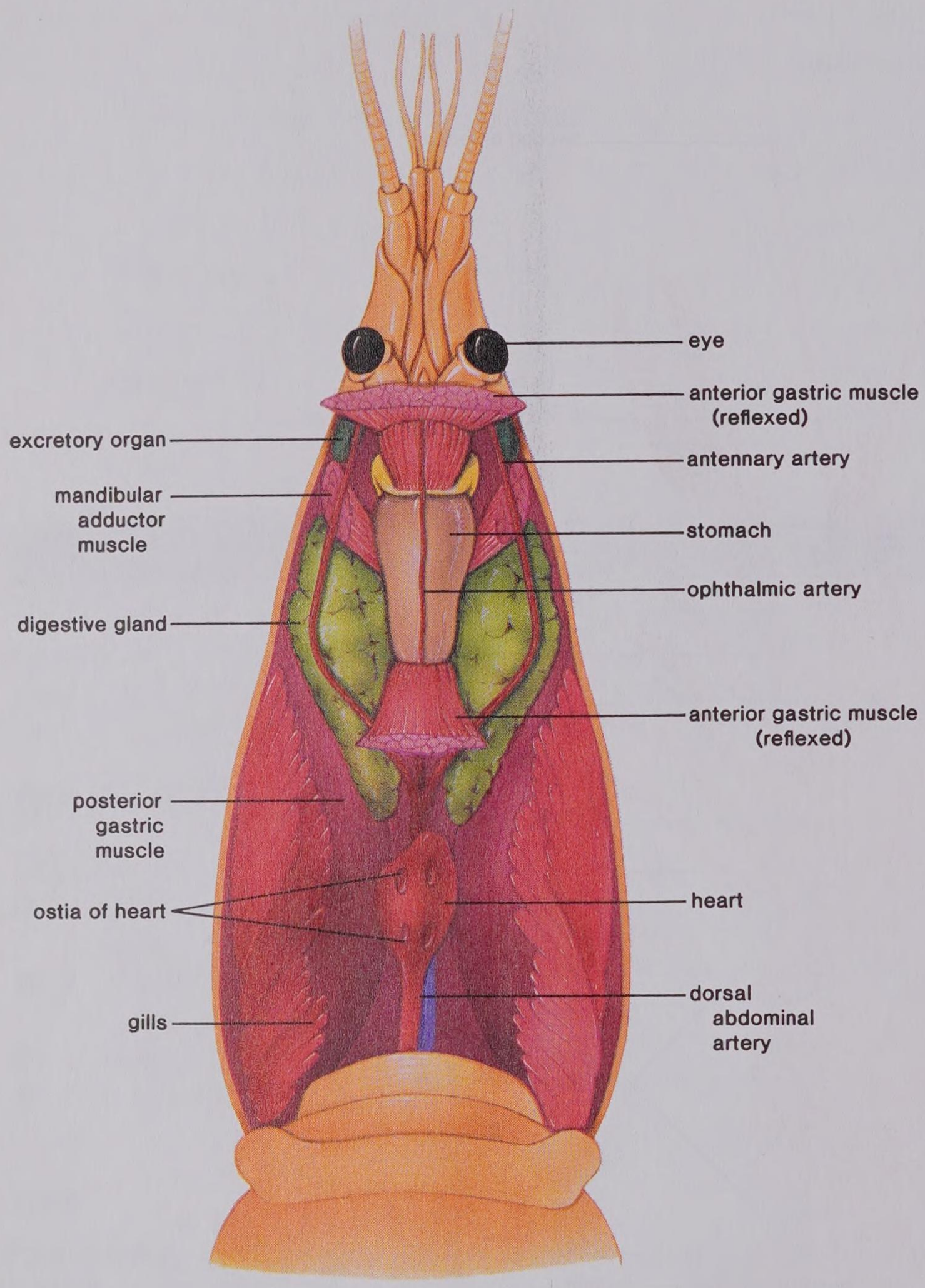
Observation of Respiration (Optional)

1. Remove the animal from the water, and cut away the *lateral* surface of the *carapace* with scissors, to expose the gills. *Note:* Consult with your *instructor* before starting this procedure.
2. After observing the movement of the gills, return the animal to the container of water.
3. Place a few drops of *India ink* near the posterior end of the chamber.

   What happens? _____

4. Lift carefully the carapace near the dorsal surface of the gill chamber to observe the beating of the heart.

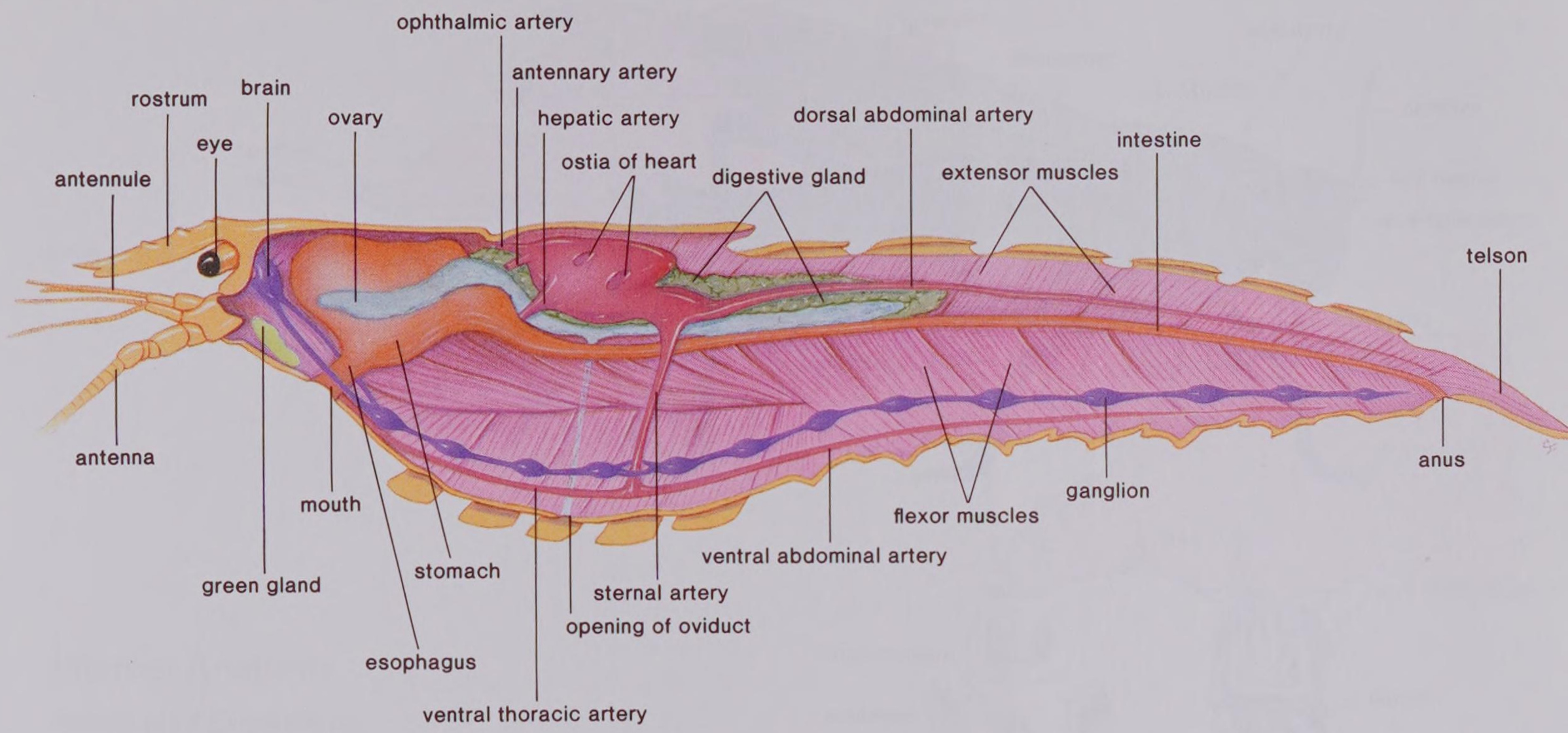
Figure 18.9 Crayfish, dorsal internal anatomy.



Internal Anatomy

- S 1. Place a preserved crayfish in a dissecting pan and pin it down.
- S 2. Add some *water* to keep the preparation moist.
- S 3. Cut away carefully the *dorsal* surface of the *exoskeleton* with scissors and a scalpel. The epidermis that adheres to the exoskeleton secretes the exoskeleton.
- S 4. Remove any epidermis lying over the external organs.
- S 5. Observe the *gill surfaces* lying in the *brachial chambers*. The first organ that you will see is the diamond-shaped *heart* lying in the middorsal region (figs. 18.9 and 18.10).
- S 6. Remove the *heart* carefully.
- S 7. Observe the *gonads* anterior to the heart in both male and female. The gonads are tubular structures bilaterally arranged in front of the heart and continuing behind it as a single mass. In the male, the testes are highly-coiled white tubes.
- S 8. Locate the *mouth,* the short tubular *esophagus,* and the *cardiac* and *pyloric stomach*. The *cardiac stomach* is used for storage; most of the digestion takes place in the *pyloric stomach*. The remainder of the digestive system is the intestine leading to the *anus*. Just anterior to the stomach are 2 excretory structures called *green glands*.

Figure 18.10 Crayfish, longitudinal section.



The nervous system is similar to that of the annelid in that there is a ventral solid nerve cord.

- 9. Keeping the digestive system intact, remove the *abdominal* and *thoracic contents* until you can locate the *brain* in front of the esophagus.
- 10. With careful dissection, find the nerves to the eyes, antennae, and antennules. The brain is connected to the ventral nerve cord by a *pair* of *nerves* that pass around the esophagus.
- 11. Now remove the entire *digestive tract* from the animal and float it in water.
- 12. Observe the various parts, especially the connections of the *digestive gland ducts* to the stomach.
- 13. Cut the *pyloric stomach* from the rest of the digestive system.
- 14. Find the *grinding stones* ingested by the crayfish. These act against the heavy toothlike projections of the stomach wall in order to grind up food. What had your specimen been eating? _____

  _____
- 15. Remove the *compound eye,* and examine under the binocular dissecting microscope. Note the *facets.*
- 16. *Sketch* the compound eye when viewed magnified by a binocular dissecting microscope or a hand lens.

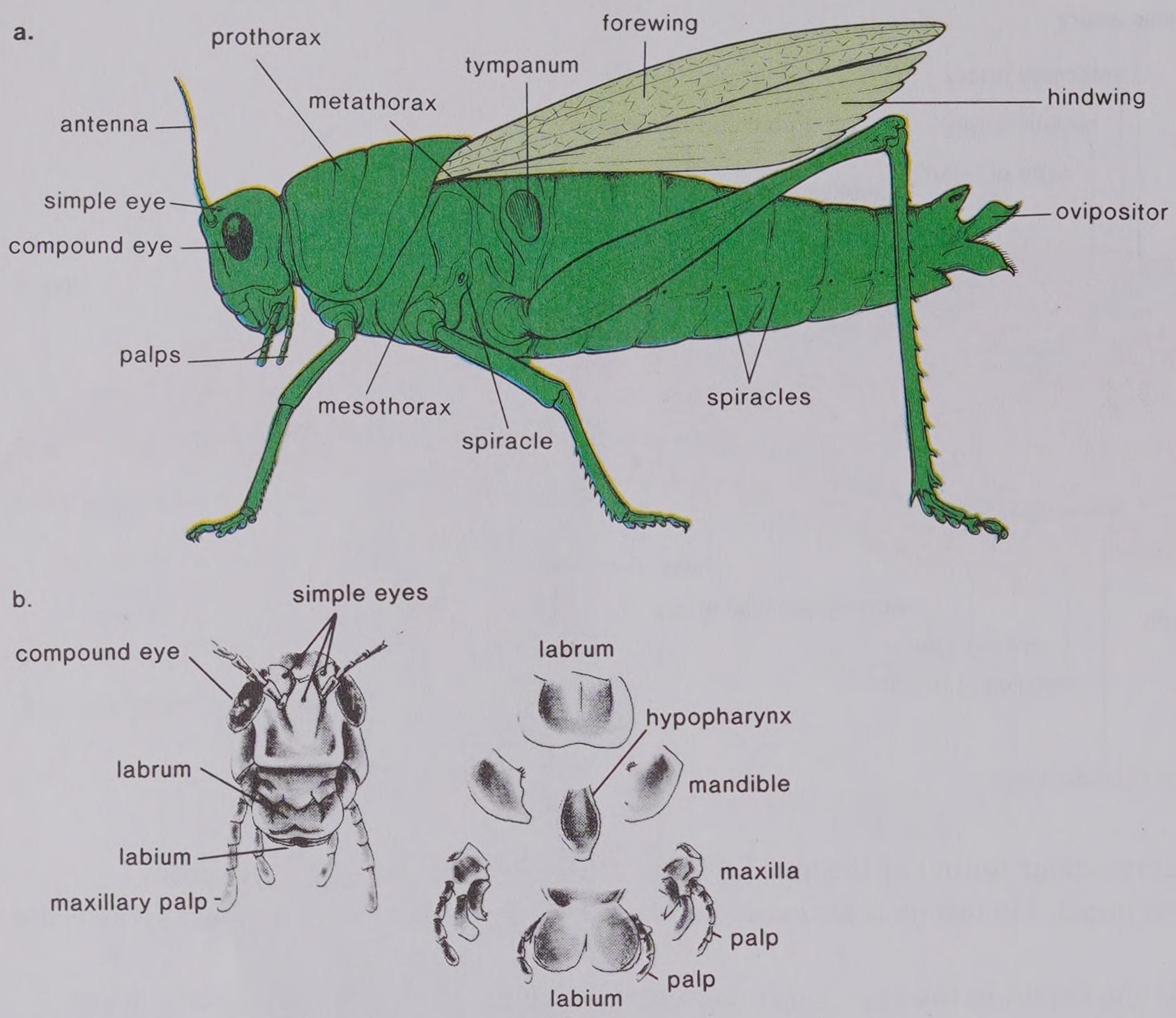
Insects (Class Insecta)

Insects are the largest class among the arthropods, with over 800,000 described species. Insects have bodies composed of the 3 distinct regions: *head, thorax,* and *abdomen.* Insects have one pair of antennae, 3 pairs of legs, and usually 2 pairs of wings. Insects are wholly adapted to life on land (even though there are some species that have aquatic adults and others that have aquatic larvae). This fact is reflected in every feature of their anatomy. We will examine the grasshopper (*Romalea*).

External Anatomy

- 1. Obtain a preserved grasshopper and study its external anatomy with the help of figure 18.11.
- 2. Identify the *head, thorax,* and *abdomen.* The *chitinous exoskeleton* consists of hardened plates separated by thin, membranous areas that allow movement of the body segments and appendages.

Figure 18.11 External anatomy of grasshopper. ***a.*** Side view. ***b.*** Head and mouthparts.



Thorax

The grasshopper's thorax consists of 3 fused segments—the large anterior *prothorax,* the middle *mesothorax,* and the hind *metathorax*. The first pair of legs is attached to the prothorax. The second pair of legs and the outer pair of straight, leathery *wings* are attached to the mesothorax. The third pair of legs and inner membranous wings are attached to the metathorax. Each leg consists of 5 segments. The hind leg is well developed and is used for jumping.

Head

S 1. Using a binocular dissecting microscope or hand lens, examine the special sense organs of the head, including the pair of long, jointed feelers, or *antennae;* the pair of large *compound eyes;* and the 3 dotlike simple eyes.

S 2. If you wish, remove the *mouthparts* by grasping them with forceps and pulling them out.

S 3. Arrange them in order on an index card and compare them to figure 18.11. These mouthparts are used for chewing and are quite different from those of a piercing and sucking insect.

Abdomen

S 1. In the abdomen, locate the *tympana,* one on each side of the first abdominal segment. By means of these membranes, the grasshopper can detect sound vibrations.

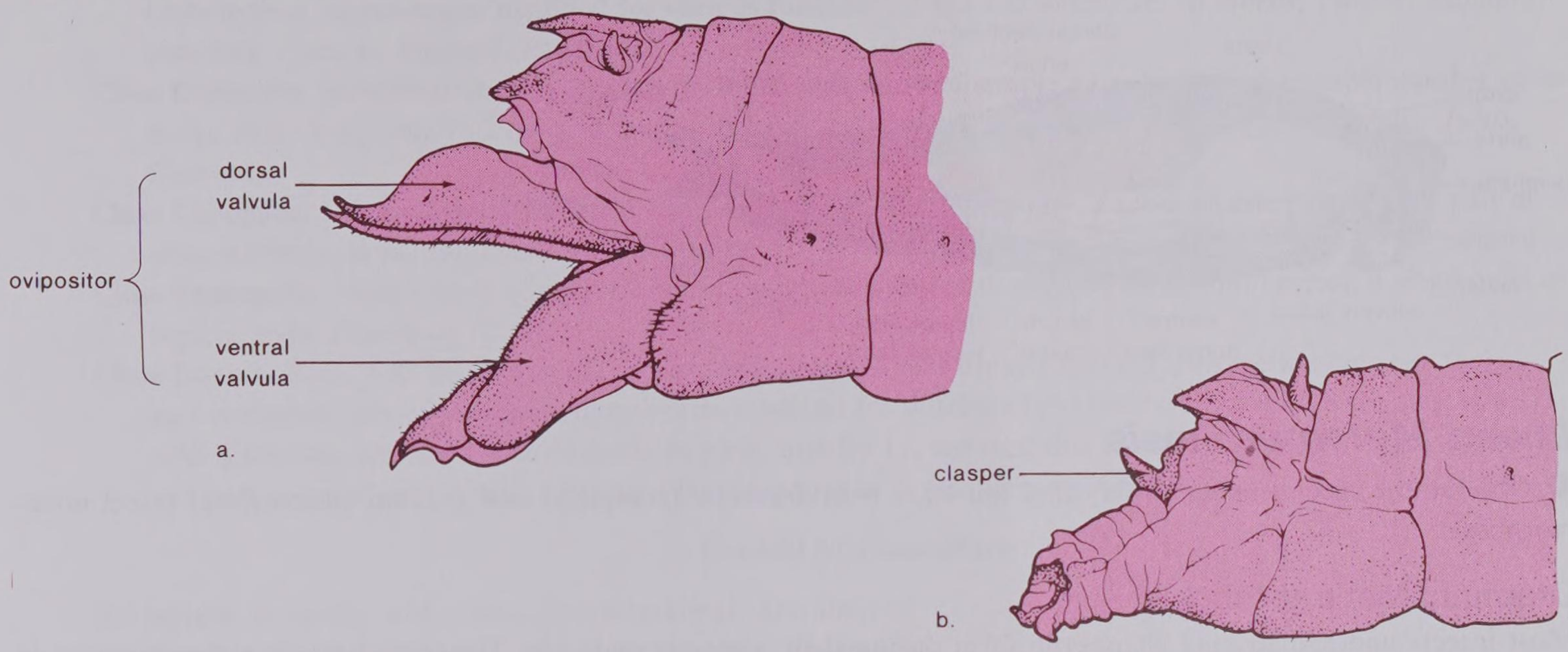
S 2. Also locate, along the sides of the abdominal segments, the *spiracles,* openings that allow air to enter the *tracheae;* these make up the respiratory system. In the *female,* 4 curved and pointed processes project from the hind end. These are the *ovipositors,* used for digging a hole in which eggs are laid (fig. 18.12).

Adaptation

The external anatomy illustrates adaptation to land in that the exoskeleton prevents water loss, the wings allow flight through air, the legs permit locomotion on land, and each tympanum detects sound waves. Can you think of any other adaptations to the land environment demonstrated by the external anatomy? _____

_____

Figure 18.12 Grasshopper genitalia. ***a.*** Female. ***b.*** Male.



Internal Anatomy

Removal of Exoskeleton

1. Detach the *wings* and *legs* of the grasshopper.
2. With the organism turned on its side, use scissors to carefully cut through the *exoskeleton* (dorsal to the spiracles) along the full length (from the head to the posterior end) of the animal.
3. Repeat this procedure on the other side.
4. Cut crosswise behind the head so that you can remove a strip of the exoskeleton.
5. If necessary, reach in with a probe to loosen muscle attachments and membranes.
6. Pin the insect to the dissecting pan dorsal side up.
7. Cover the specimen with water to keep the tissues moist.

Organs

The *heart* (fig. 18.13) and a longitudinal artery should be visible just beneath the portion of exoskeleton you removed. Blood leaves the circulatory system to enter the *hemocoel,* a network of open spaces where blood flows about the organs.

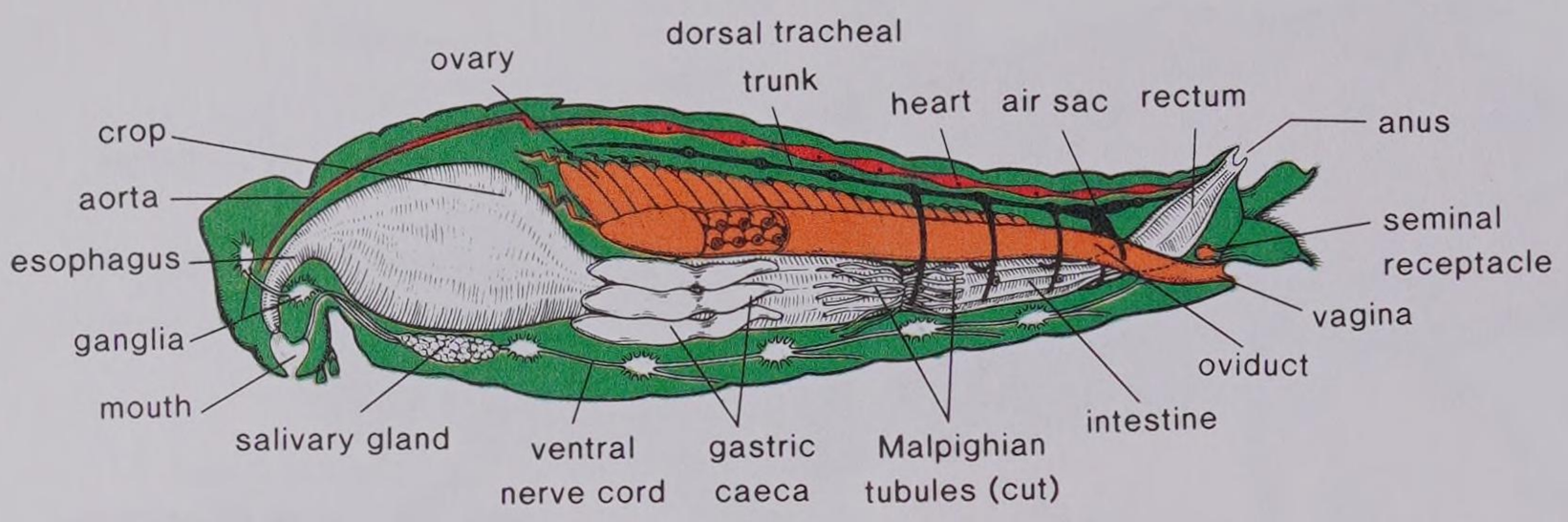
1. Remove the heart and adjacent tissues to expose the *fat body,* a yellowish fatty tissue that covers the internal organs.
2. Carefully remove the fat body. With the help of the dissecting microscope, see if you can detect the glistening white tubules of the *tracheal system,* the respiratory system of insects.
3. Notice the *reproductive organs* that lie on either side of the alimentary canal in the abdomen.
4. Separate these into the left and right ovaries (or testes). Each *ovary* is essentially a collection of parallel, tapering tubules containing cigar-shaped eggs. Each *testis* is a coiled, elongated cord containing many tubules.
5. Trace the alimentary canal beginning with the *crop* (fig. 18.13), a large pouch.
6. Next, note the *gastric caeca* that are attached to the *stomach,* and the *intestine* with its numerous *Malpighian tubules.* These are the excretory organs.
7. Work the *alimentary canal* free and move it to one side.
8. Observe the *salivary glands* that extend into the thoracic cavity.
9. Remove all the internal organs to expose the ventral *nerve cord,* which is thickened at intervals by ganglia. To observe the *brain,* you will have to very carefully remove one side of the exoskeleton covering the head.

Adaptation

The internal anatomy illustrates adaptation to land in that the tracheae conduct air to all parts of the body; the digestive system is modified to process vegetation; and the Malpighian tubules excrete uric acid, a nitrogenous waste that is excreted dry. Can you think of any other adaptations to land demonstrated by the internal anatomy? _____

_____

Figure 18.13 Internal anatomy of grasshopper.



Insect Metamorphosis

Observe the specimens on display that illustrate both *dramatic* (complete) and *gradual* (incomplete) insect metamorphosis.

Dramatic Metamorphosis

Most insects undergo striking changes in form during their postembryonic life. The characteristic series of stages in complete metamorphosis include the following: *egg, larva, pupa,* and *adult*. The egg hatches into a segmented, wormlike larva. The larva feeds, grows, undergoes several molts; after the final molt, transforms into a pupa. The pupa is a stationary, nonfeeding stage. The pupa appears to be inactive, but actually the larval tissues are disappearing as the adult organs are being formed. When the adult is fully formed, the pupal case is ruptured, and the adult emerges.

Name 4 insects (each from different orders) that illustrate dramatic (complete) metamorphosis.

_____ _____

_____ _____

Gradual Metamorphosis

The grasshopper is an excellent example of gradual metamorphosis.

Observe the *eggs* and several *nymph* stages and compare each of them with the *adults*. Note especially the changes in the head-body proportions and the gradual development of the wings and sexual maturity.

Using the arthropod classification list following, name 4 insects (each from different orders) that illustrate gradual (incomplete) metamorphosis.

_____ _____

_____ _____

Arthropod Classification (Partial and Simplified)

Subphylum Trilobita. No living forms.

Subphylum Chelicerata. First pair of appendages modified to form chelicerae (pincerlike head appendages) with claws; pair of pedipalps on head and 4 pairs of legs; no antennae; no mandibles; cephalothorax and abdomen usually nonsegmented.

Class Arachnida. Four pairs of legs; segmented or unsegmented abdomen with or without appendages and generally distinct from cephalothorax; respiration by gills, tracheae, or book lungs (air pockets in blood-filled chamber). **Scorpions, spiders, mites, ticks, harvestmen.** *Archaearanea Latrodectus, Argiope, Centruroides, Chelifer, Mastigoproctus, Phalangium, Ixodes.*

Subphylum Mandibulata. Head appendages consisting of one or two pairs of antennae, one pair of mandibles, and one or 2 pairs of maxillae.

Class Crustacea. Mostly aquatic, with gills, hard exoskeleton; cephalothorax with dorsal carapace; biramous (2-branched) appendages, modified for various functions; 2 pairs of antennae. *Homarus, Cancer, Daphnia, Artemia, Cyclops, Balanus, Porcellio.*

Class Diplopoda. Subcylindric body; head with short antennae and simple eyes; body with variable number of somites; short legs, usually 2 pairs of legs to a somite. **Millipedes.** *Narceus, Apheloria, Polydesmus, Julus, Glomeris.*

Class Chilopoda. Dorsoventrally flattened body; variable number of somites, each with one pair of legs; pair of long antennae, jaws, and maxillae. **Centipedes.** *Scolopendra, Lithobius, Scutigera.*

Class Pauropoda. Minute (1–1.5 mm); cylindric body consisting of double segments and bearing 9 or 10 pairs of legs; no eyes. *Pauropus.*

Class Insecta. Body with distinct head, thorax, and abdomen and usually marked constriction between thorax and abdomen; pair of antennae; mouthparts modified for different food habits; head with 6 somites; thorax with 3 somites; abdomen with variable number, usually 11, somites; thorax with 2 pairs of wings (sometimes one pair or none) and 3 pairs of jointed legs; gradual or dramatic metamorphosis.

Gradual Metamorphosis

Springtails. *Isotoma, Achorutes, Neosminthurus, Sminthurus*
Bristletails, silverfish, firebrats. *Machilis, Lepisma, Thermobia*
Mayflies. *Hexagenia, Callibaetis, Ephemerella*
Dragonflies, damselflies. *Archilestes, Lestes, Aeshna, Gomphus*
Grasshoppers, crickets, walking sticks, mantids, cockroaches. *Schistocerca, Romalea, Nemobius, Megaphasma, Mantis, Blatta, Periplaneta*
Termites. *Reticulitermes, Kalotermes, Zootermopsis, Nasutitermes*
Earwigs. *Labia, Forficula, Prolabia*
Stoneflies. *Isoperla, Taeniopteryx, Capnia, Perla*
Book lice. *Ectopsocus, Liposcelia, Trogium*
Chewing lice. *Cuclotogaster, Menacanthus, Menopon, Trichodestes*
Sucking lice. *Pediculus, Phthirius, Haematopinus*
True bugs. *Belostoma, Lygaeus, Notonecta, Cimex, Lygus, Oncopeltus*
Cicadas, aphids, leafhoppers, scale insects. *Magicicada, Circulifer, Psylla, Aphis, Saissetia*

Dramatic Metamorphosis

Dobsonflies, alderflies, lacewings, antispids, snakeflies. *Corydalus, Hemerobius, Chrysopa, Mantispa, Agulla*
Beetles, weevils. *Copris, Phyllophaga, Harpalus, Scolytus, Melanotus, Cicindela, Dermestes, Photinus, Coccinella, Tenebrio, Anthonomus, Conotrachelus*
Wasps, bees, ants, sawflies. *Cimbex, Vespa, Glypta, Scolia, Bembis, Formica, Bombus, Apis*
Scorpionflies. *Panorpa, Boreus, Bittacus*
Fleas. *Pulex, Nosopsyllus, Xenopsylla, Ctenocephalides*
True flies, mosquitoes. *Aedes, Asilus, Sarcophaga, Anthomyia, Musca, Chironomus, Tabanus Tipula, Drosophila*
Caddisflies. *Limnephilus, Rhyacophila, Hydropsyche*
Moths, butterflies. *Tinea, Pyrausta, Malacosoma, Sphinx, Samia, Bombyx, Heliothis, Papilio, Lycaena*

Survey of Arthropods

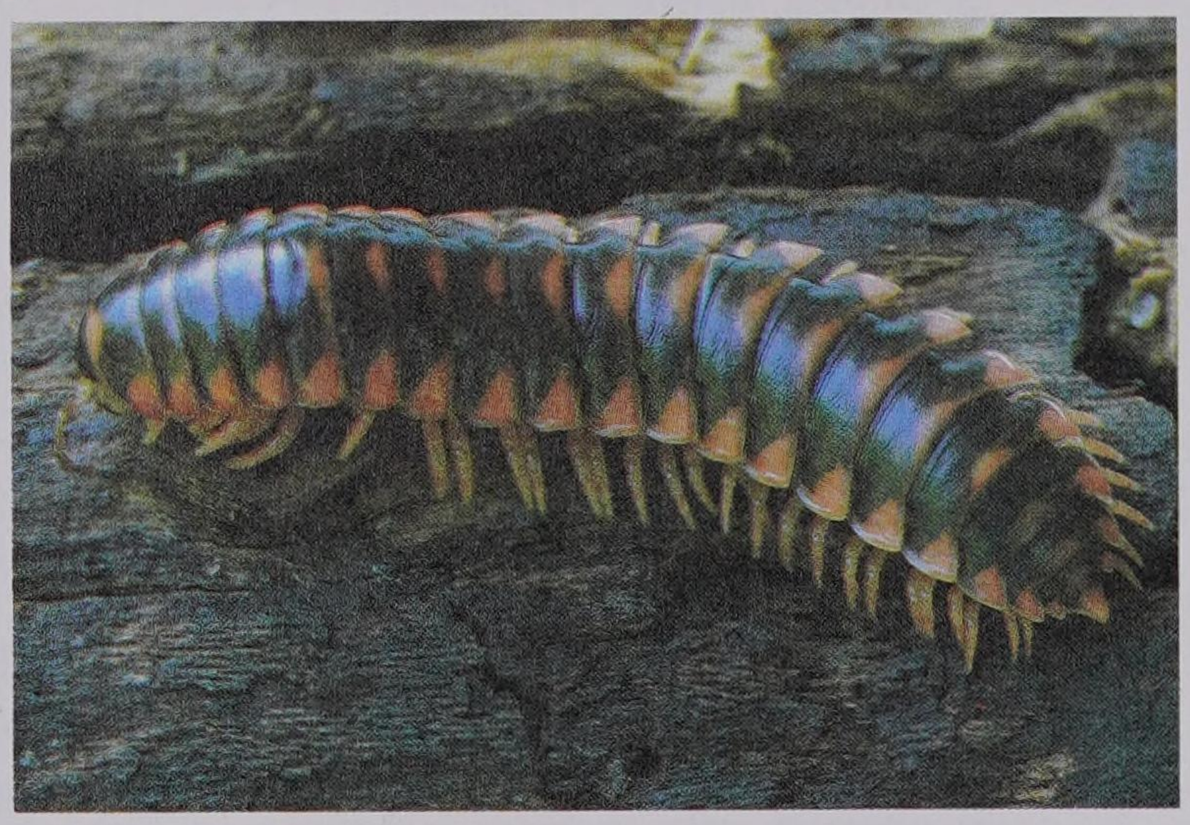
1. On display are a number of arthropod animals. *Study* each one of these carefully.
2. Fill in table 18.1 and using one example for each class represented. See figure 18.14 and the preceding arthropod classification.
3. First, give the *common name* of the organism; second, state one *general arthropod feature* it has; third, name the class; and fourth, write one *specialized feature* that makes this organism a member of this class.

Figure 18.14 There are 5 major classes of Arthropods. ***a.*** Class Crustacea is represented by this crab with a large carapace and 5 pairs of legs. The first pair are pinching claws. ***b.*** Class Arachnida is represented by this land-dwelling scorpion, with 4 pairs of legs, poisonous claws, and stinging tail. ***c.*** Class Chilopoda is represented by this centipede, a carnivorous animal, with a pair of appendages on every segment. ***d.*** Class Diplopoda is represented by this millipede, a scavenger that seems to have 2 pairs of appendages on each segment because every 2 segments are fused. Class Insecta is represented by ***e.*** a swallowtail butterfly with wings and ***f.*** an ant with clearly defined head, thorax, and abdomen. Insects have 3 pairs of legs.

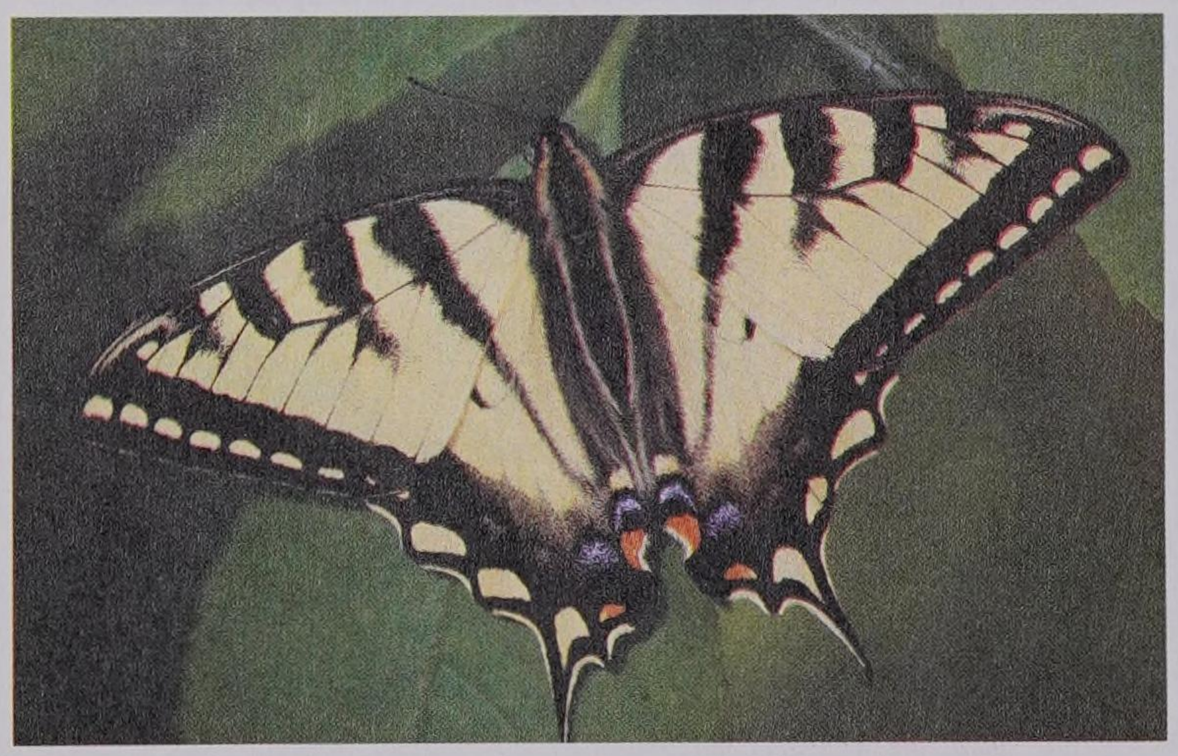
a.

b.

c.



d.



e.

f.

Table 18.1 Survey of Arthropods

Common Name	Arthropod Features	Class	Specialized Features

For each of the characteristics listed, explain what is *visible* (internal or external) in the structure of the arthropods that illustrates each characteristic, and explain its *usefulness* to the organism. Also indicate (using the letter abbreviations) if each characteristic is primitive (P), advanced (A), or most advanced (MA). Refer to table 17.1 if necessary.

Bilateral symmetry with cephalization _____

_____

_____

Segmented with specialization _____

_____

_____

_____

Laboratory Review 18

2 1. All mollusks have a foot, a visceral mass, and a _____ .

2 2. Is a clam adapted for an active or inactive life?

2 3. What type of circulatory system is found in the clam? in the earthworm?

2 4. Name 2 internal organ systems you observed in an earthworm that show segmentation.

_____

2 5. Is segmentation found in arthropods?

2 6. Do segmented animals usually show more or less specialization of parts than nonsegmented animals?

2 7. Name 2 advantages of having a skeleton.

_____

2 8. How do insects breathe?

9. What is the defining characteristic of a protostome?

Laboratory

19

Diversity VI: Deuterostomes

Learning Objectives

Students should be able to

1. identify and classify the animals studied in this laboratory;
2. identify the anatomical parts studied in the laboratory and state the function of these.

Introduction

Animals are often grouped by the type of body cavity they have. The so-called primitive vertebrates are acoelomate (no cavity) or pseudocoelomate (false cavity). Some *protostomes* (mollusks, annelids, arthropods) and all *deuterostomes* are true *coelomates* (true body cavity lined by mesoderm). In protostomes, the first opening of the embryo becomes the mouth. In this lab, we will study the deuterostomes.

The deuterostomes are a second branch of the evolutionary tree of animals (fig. 17.1). These animals are termed *deuterostomes* because the first opening of the embryo becomes the *anus* and a second (deutero) opening becomes the *mouth* (stoma).

The deuterostomes comprise only 2 phyla: the *echinoderms* and the *chordates*. The largest group of chordates are the *vertebrates,* the most advanced of all the animals. It is curious that the chordates are believed to be related to the echinoderms, who as adults have primitive features including radial symmetry. These animals do have bilateral symmetry as larvae, however, and embryological evidence suggests that they are related to the chordates.

Echinoderms (Phylum Echinodermata)

The echinoderms are exclusively marine organisms, possessing *spiny skin, radial symmetry* as adults, and a *water vascular system* that provides a means of locomotion. This phylum includes *sea stars* (starfishes), *sea urchins, sand dollars, sea cucumbers,* and *sea lilies.*

Sea Star (Class Asteroidea)

External Anatomy

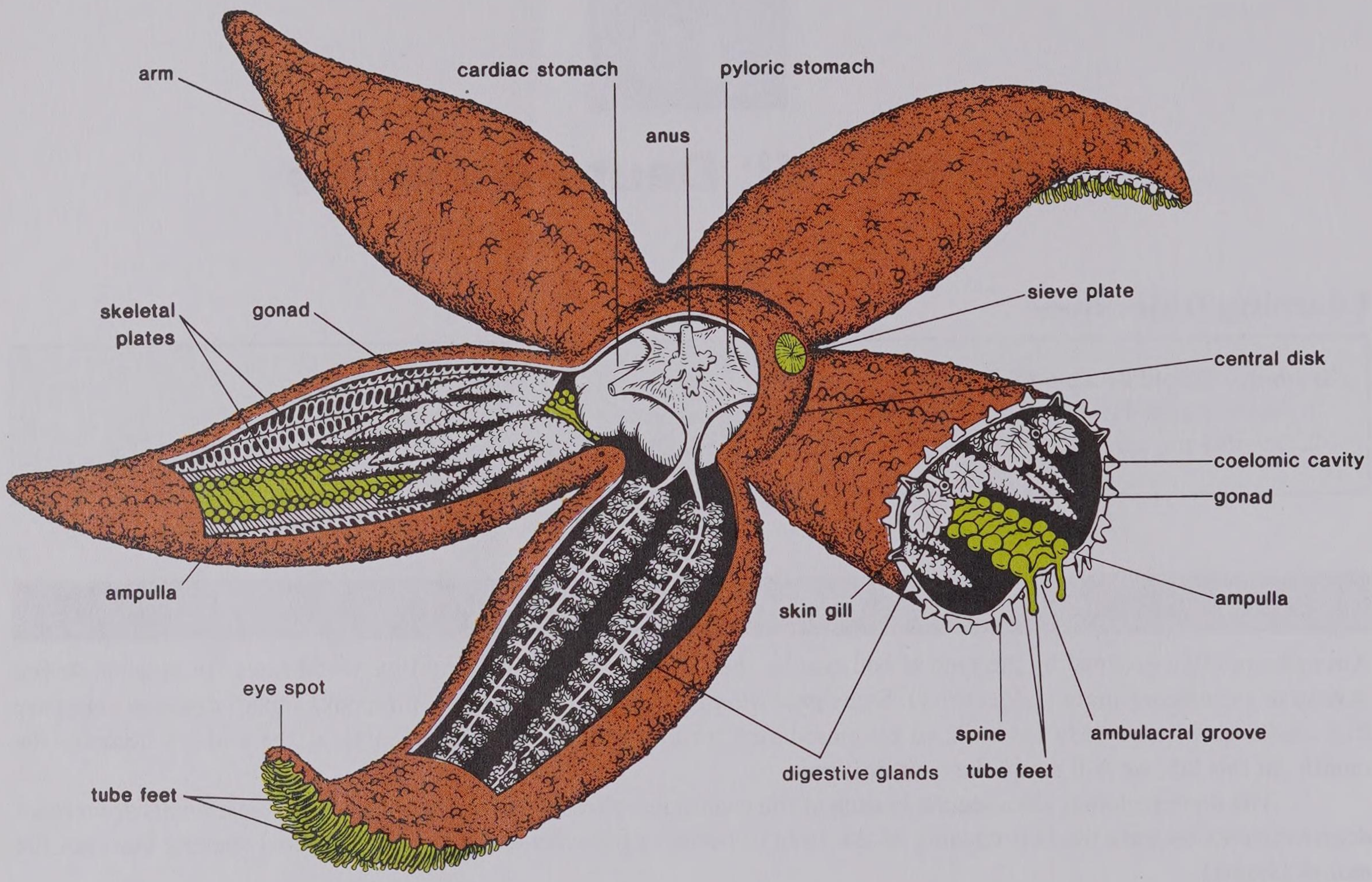
Ⓢ 1. Place a preserved sea star in a dissecting pan, and note externally the *central disc* and *5 arms* (fig. 19.1). On the upper side locate the sieve plate (*madreporite*), a brownish circular spot between 2 arms that leads to the system of tube feet. This side of a sea star is called the *aboral side,* and the anal opening is found on this side. The *spines* of the aboral surface are parts of small, calcareous *endodermal plates* that lie buried in the wall beneath the surface. These plates form the *endoskeleton* of this animal.

Ⓢ 2. Turn the sea star over.

Ⓢ 3. On the *oral* surface, locate the central *mouth* protected by spines. Running along the middle of each arm is an *ambulacral groove,* studded with *tube feet.*

Ⓢ 4. Pluck away the tube feet from one area, and determine how many rows of feet there are. In the valley of each ambulacral groove, identify the *radial canal* extending to the tip of each arm.

Figure 19.1 Starfish anatomy.



Internal Anatomy

Ⓢ 1. Place the sea star so that the *aboral* side is uppermost (fig. 19.1).
Ⓢ 2. Cut the tip of one of the arms and, with scissors, cut carefully through the body wall along each side of this arm.
Ⓢ 3. Cautiously lift up the upper body wall. Separate any internal organs that may be adhering and leave all internal organs intact.
Ⓢ 4. Cut off the body wall near the central disc but leave the *sieve plate* in place.
Ⓢ 5. Remove the body wall of the central disc, making sure you do not injure the internal organs.

Note the 2-part stomach, the *saclike cardiac stomach* and the narrower *pyloric.* A short *intestine* is present. Each arm contains one pair of *digestive glands,* which connect to the pyloric stomach.

Ⓢ 6. Remove the glands of one arm and locate the pair of feathery *gonads* extending into the arm.

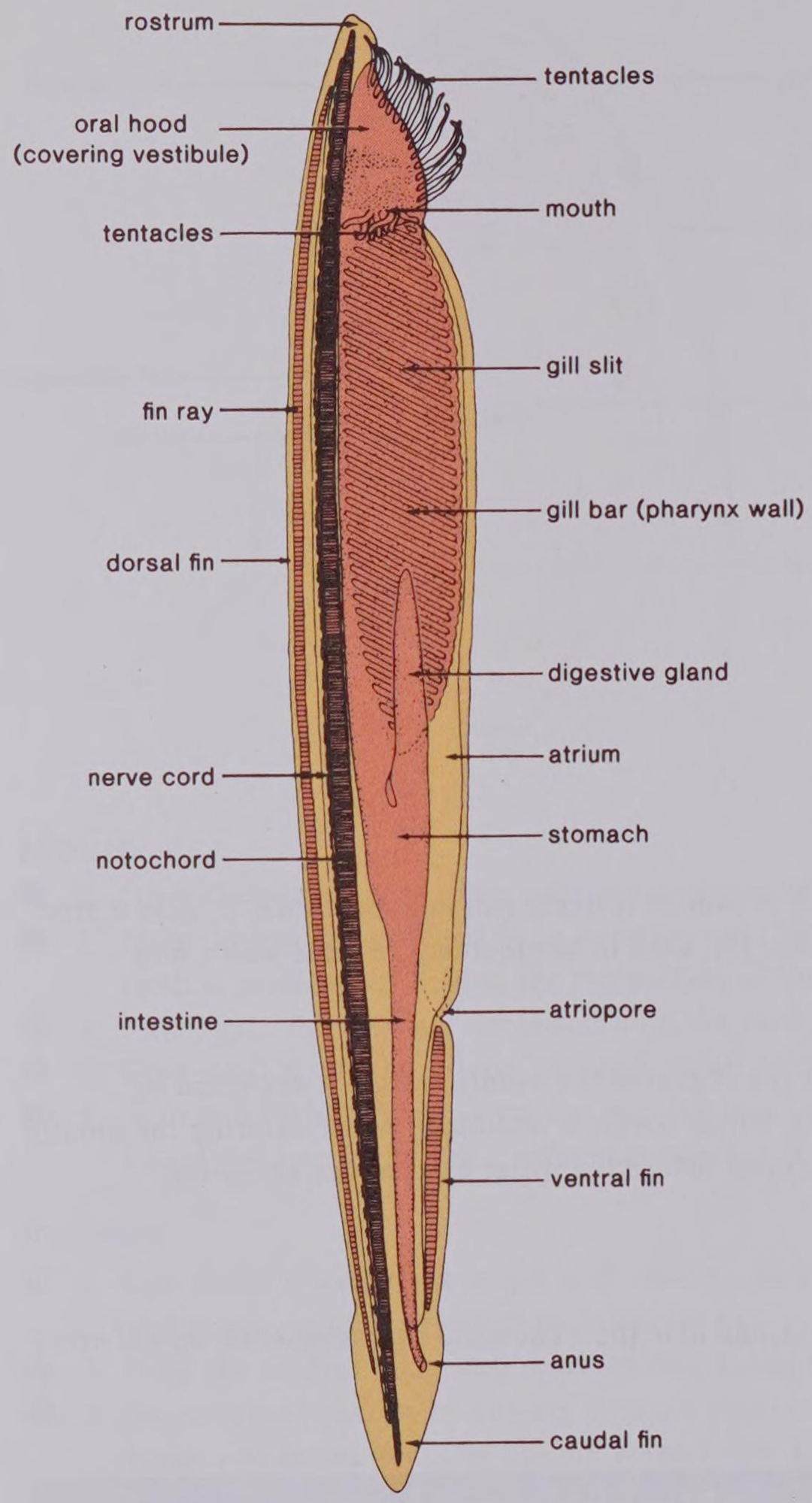
Water Vascular System

The *sieve plate* on the aboral surface is the entrance to the closed water vascular, or locomotor, system. Only echinoderms have such a system. Though covered by the stomach in figure 19.1, you may move it aside the stomach.

Ⓢ Locate the *stone canal,* which connects the madreporite with the *ring canal* that surrounds the mouth opening. From the ring canal, the radial canals extend into each arm. The *radial canals* may be found within the groove that runs the length of each arm.

Transverse canals lead from each radial canal to the *ampullae* of the tube feet. Each tube foot is a cylindrical tube ending in a suctionlike disc at one end and a rounded body, the ampulla, at the other. As the ampulla contracts and forces water into the foot, longitudinal muscles in the tube feet contract to swing the foot forward, and longitudinal muscles on the other side of the tube foot contract once the end of the foot attaches to the substrate, pulling the animal forward. The alternate action of large numbers of tube feet brings about the slow movements characteristic of these animals.

Figure 19.2 Lancelet anatomy.



Cross Section of an Arm

1. Cut off a portion of an arm and examine the cut edge (fig. 19.1).
2. Locate the *digestive glands, ambulacral groove, radial canal, ampulla,* and *tube feet.* All these structures lie within the *coelomic cavity.* Echinoderms have a true coelom.

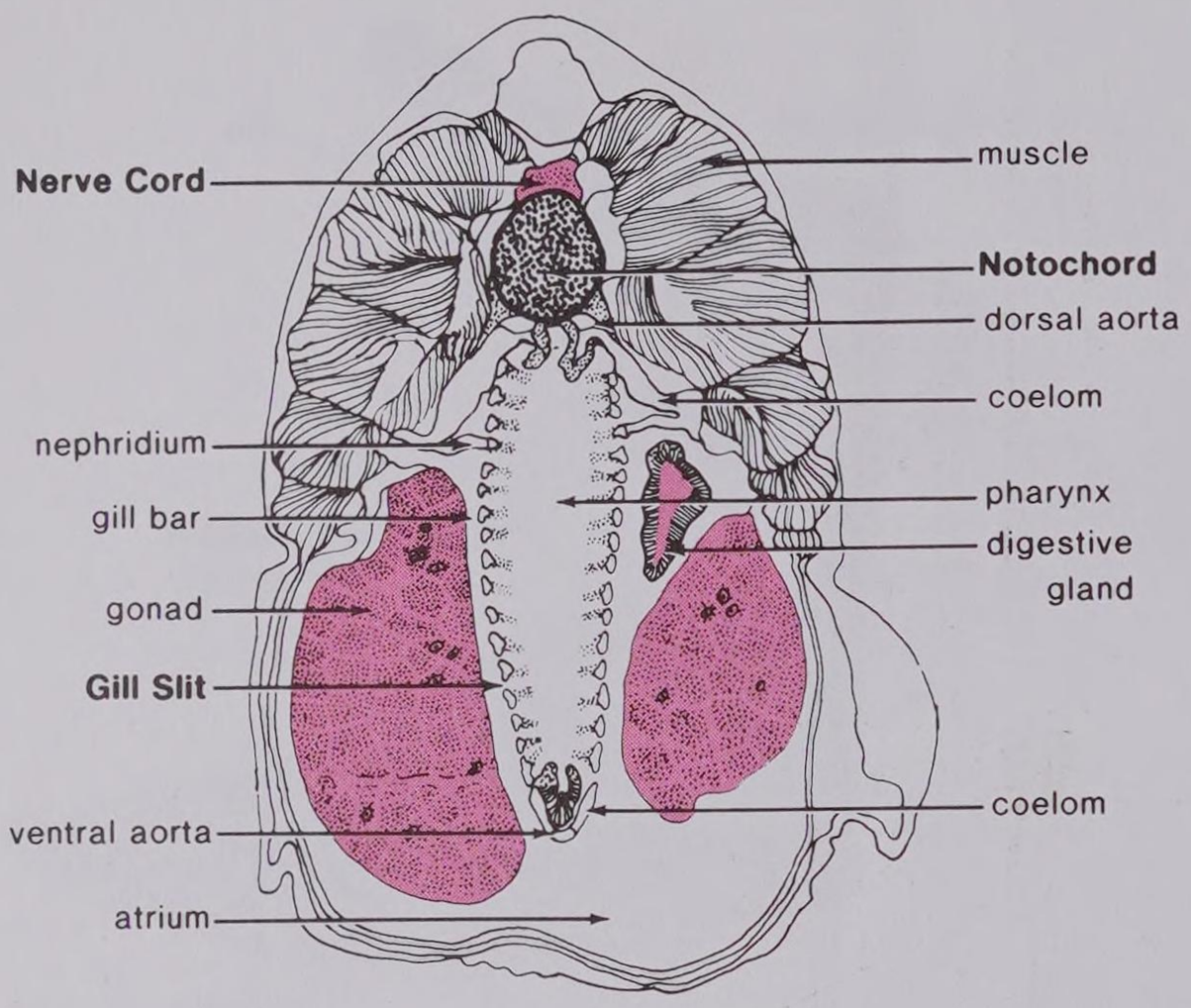
Chordates (Phylum Chordata)

The phylum Chordata contains the most highly developed organisms in the animal kingdom. All chordates possess a supporting rod called the *notochord, pharyngeal gill pouches,* and a *dorsal tubular nerve cord* sometime during their life.

Lancelet (Subphylum Cephalochordata)

Lancelets (amphioxus), the most commonly studied invertebrate chordates, are small, fishlike animals that occur in shallow marine waters in most parts of the world (fig. 19.2). They spend most of their time buried in the sandy bottom with only the anterior end projecting.

Figure 19.3 Lancelet cross section as it would appear under the microscope.



Preserved Specimen

Ⓢ 1. Examine a preserved specimen of a lancelet. Although this organism is like a fish in appearance, it lacks a true head and has no vertebrae. Note the enlarged tail, or *caudal fin,* used in locomotion. There is also a long *dorsal fin* and a short *ventral fin.*

Ⓢ 2. Observe the V-shaped *muscles* on the sides of the animal.

Ⓢ 3. Anterior to the ventral fin, note 2 folds of skin that end in the region of the *mouth,* which is encircled by *tentacles.* Anterior to the mouth is the tentacled *oral hood,* which covers a *vestibule.* Water entering the mouth is channeled into the pharynx, where food particles are trapped before the water exits at the *atriopore.* Lancelet is a filter feeder.

Three Chordate Characteristics

Ⓢ Examine a prepared cross section slide of a lancelet (amphioxus), and note the 3 chordate characteristics: *dorsal nerve cord, notochord,* and *gill slits* (fig. 19.3).

Vertebrates (Subphylum Vertebrata)

The frog may be conveniently used as a representative of the subphylum vertebrata.

Frog (Class Amphibia)

External Anatomy of Frog

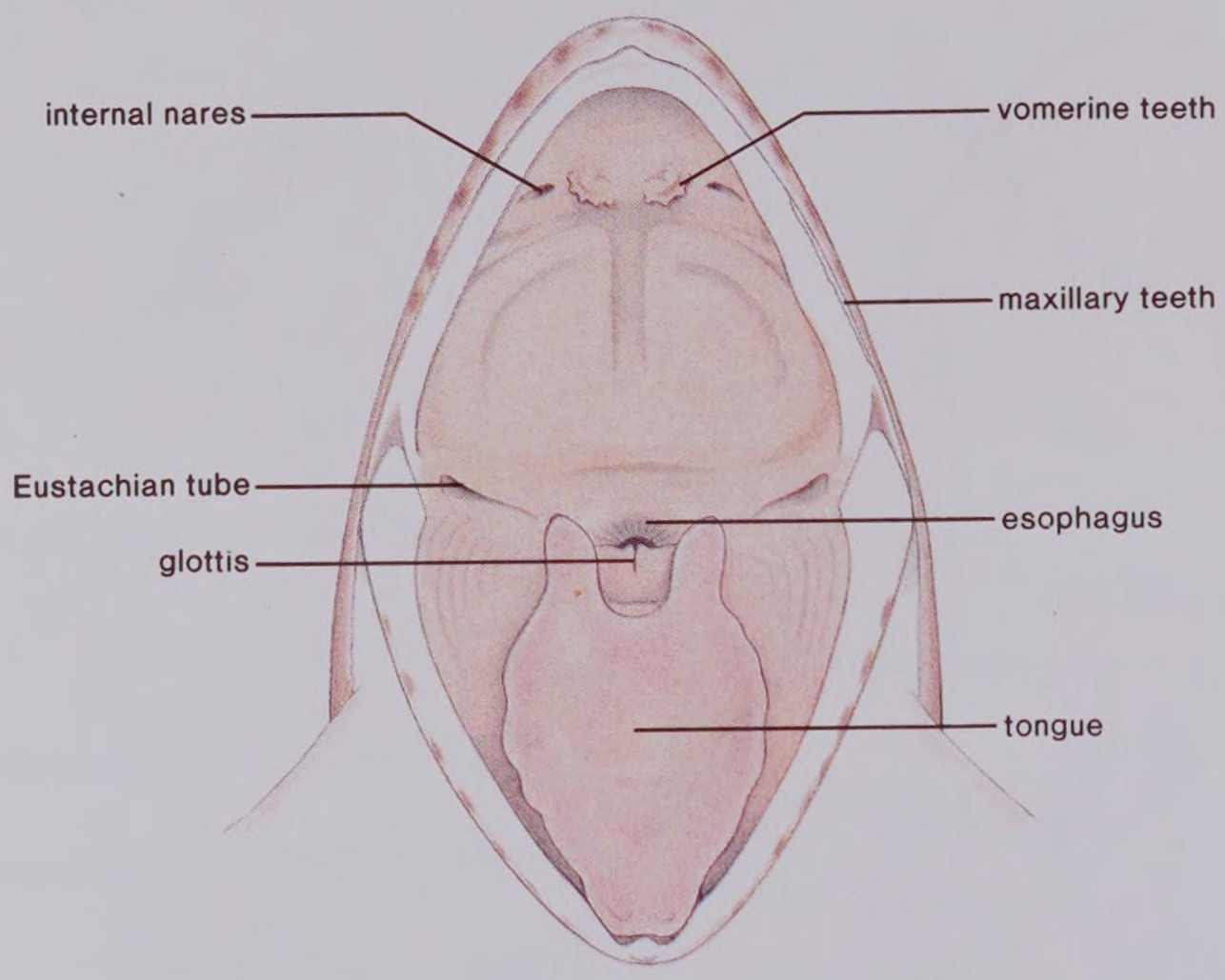
Head and Trunk

Ⓢ 1. Identify the *head* and *trunk.* The sense organs include the *nostrils, eyes,* and *ears.* The 2 openings just posterior to the snout are the *external nares* (nostrils).

Ⓢ 2. Insert a probe into one and observe that it protrudes from one of the pair of small openings, *internal nares,* inside the mouth cavity. The bulging eyes have a nonmovable upper and lower lid but can be covered by a *nictitating membrane.* The latter is attached to the inside of the lower lid. Behind each eye is the *eardrum,* or tympanum.

Ⓢ Observe the paired appendages. The short forelimb consists of an *upper arm,* a *forearm,* and a *hand.* The hand has 4 digits and a vestigial thumb. The hindlimb consists of a *thigh,* a *shank,* and a *foot.* The foot has 5 digits and a rudimentary sixth digit.

Figure 19.4 Frog, mouth cavity.



Mouth

1. Open the *mouth* very wide, cutting the angles of the jaws if necessary (fig. 19.4).
2. Note the *tongue* attached to its anterior end. Along the rim of the upper jaw feel the *teeth*. Another set of teeth is present just behind the midportion of the upper jaw.
3. Find again the *internal nares* and note the *pharynx* (throat).
4. Find there a slit, the *glottis,* through which air passes into and out of the larynx (voice box).
5. Also find within the pharynx the opening into the *esophagus* leading to the stomach. In the angles of the jaws, locate the opening of the *Eustachian tubes,* which communicate with the ears.

Incision

1. Lift the skin with forceps and with scissors make a large circular cut, taking the skin from the ventral abdominal region passing close to the limbs.
2. Note the ventral body wall *muscles* and, along the midventral line, the abdominal vein.
3. Remove the muscles by cutting through them in the same circular fashion. At the same time, cut through any bones you encounter. The *coelom* should now be exposed.

Removal of Ovary in Females

If you have a female frog, much of the coelom is likely to be filled by a pair of large transparent *ovaries* (fig. 19.7), each containing hundreds of black-and-white *eggs*.

Gently lift the left *ovary* with forceps and find its place of attachment. Cut through the attachment and remove the ovary in one piece.

Internal Anatomy of Frog

Respiratory System

Insert a probe into the *glottis* and observe its passage into the *larynx* and *trachea*. Two short *bronchi* lead to the *lungs*. The lungs are probably partly hidden under the *liver,* the large, prominent dark-brown organ in the midventral portion of the trunk.

Circulatory System

1. To find the *heart,* you may have to shift the liver gently. The heart is covered by a membranous covering.
2. Lift it and slit open this covering.
3. Identify the single thick-walled *ventricle* and the 2 more anterior, thin-walled *atria*.
4. Lift the heart and, if necessary, snip the slender strand of tissue that connects the atria to the membrane.

Figure 19.5 Internal frog anatomy.

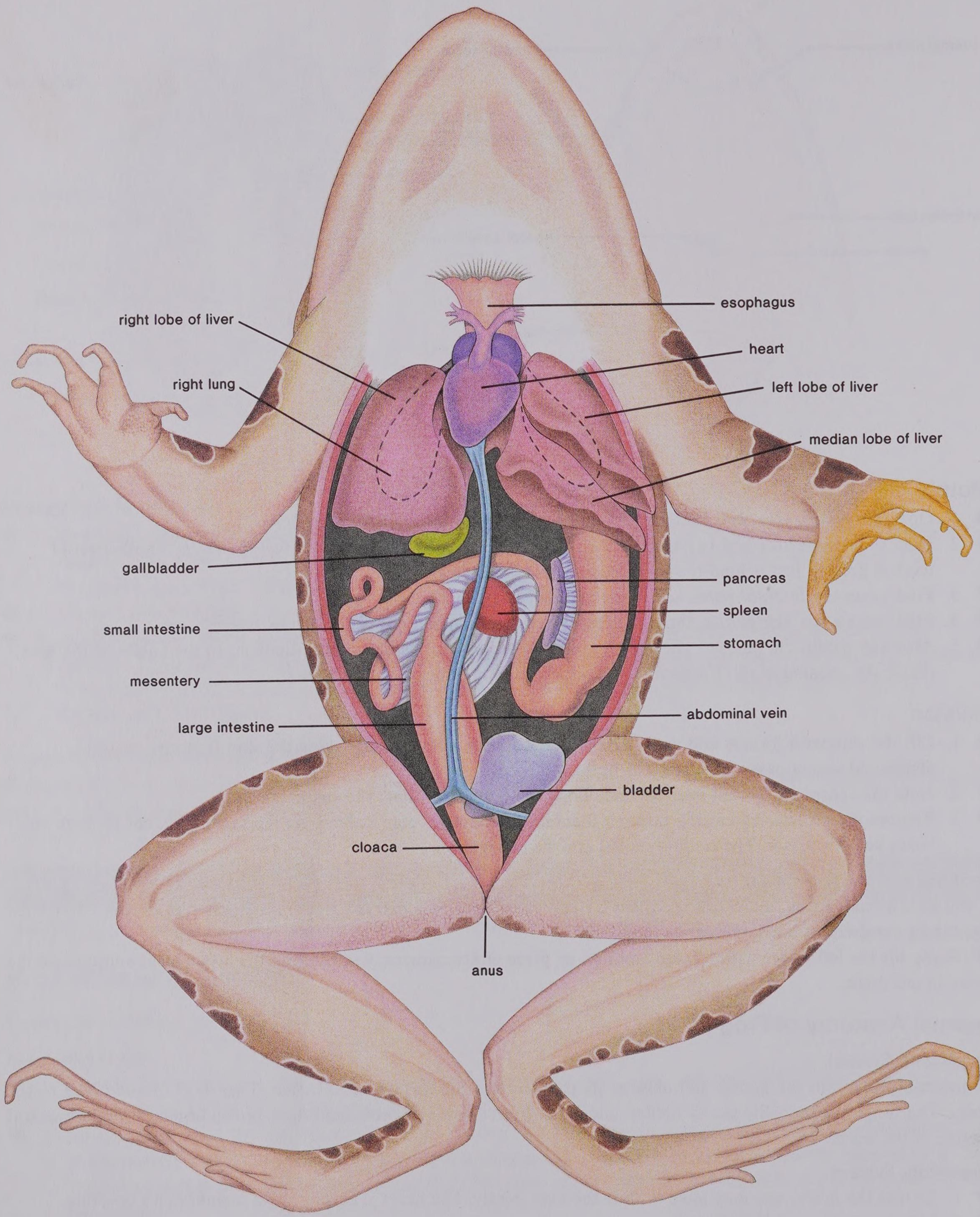
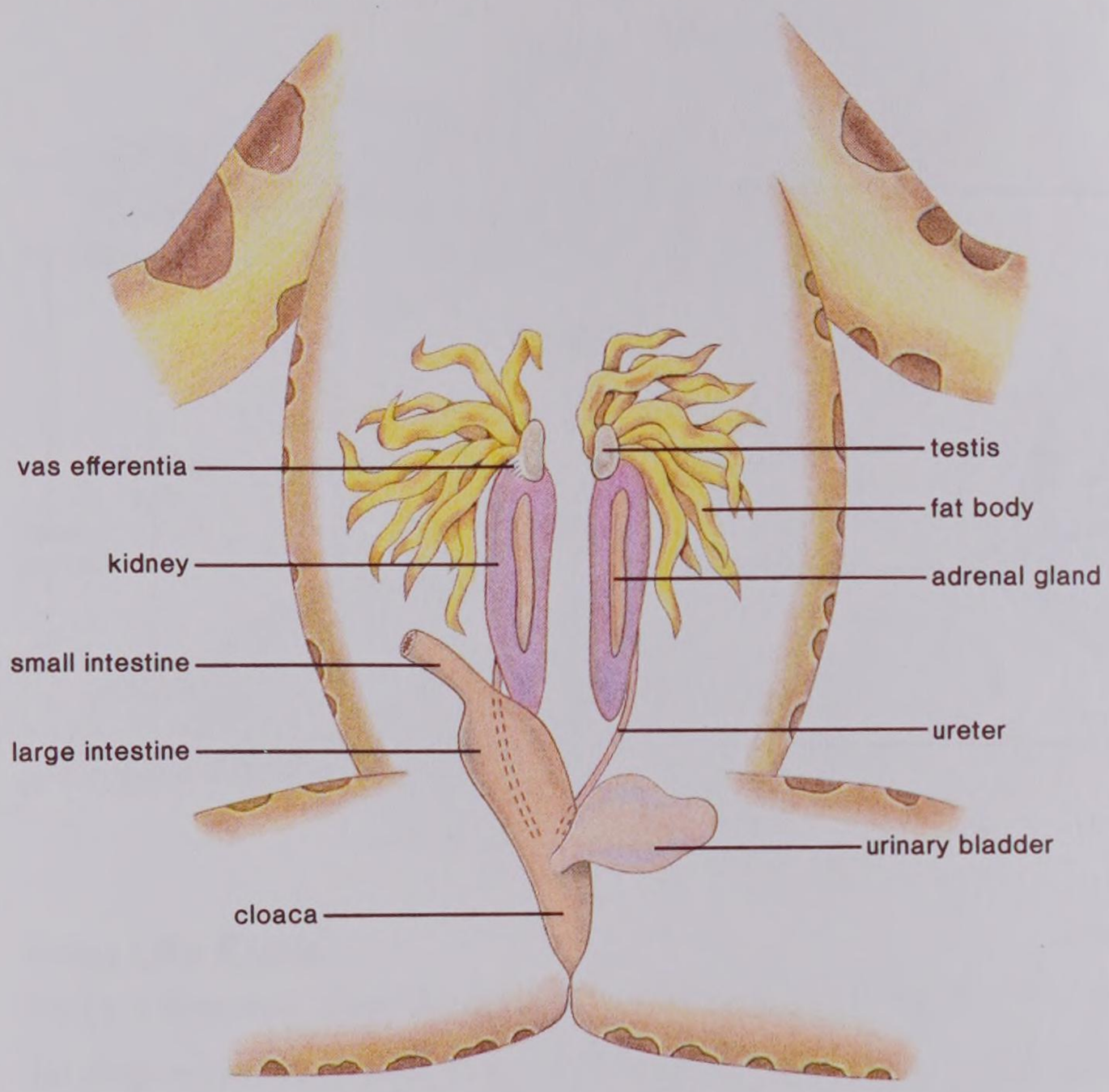


Figure 19.6 Frog, male urogenital system.



5. Note 3 large veins that join together beneath the heart to form the *sinus venosus*. Blood from the sinus venosus enters the right atrium. The left atrium receives blood from the lungs.
6. Let the heart fall back to its normal position and locate the *conus anteriosus,* a single, wide arterial vessel leaving the ventricle and passing ventrally over the right atrium.
7. Follow it forward to where it divides into 2 branches. Each branch of the conus arteriosus divides into 3 arteries. The middle artery is the *systemic artery.* Follow this vessel as it arches to the *dorsal aorta,* a large vessel that may contain a red dye.
8. Locate in the same region the *posterior vena cava,* which may contain a blue dye.

Which vessel lies above (dorsal) to the other? _____

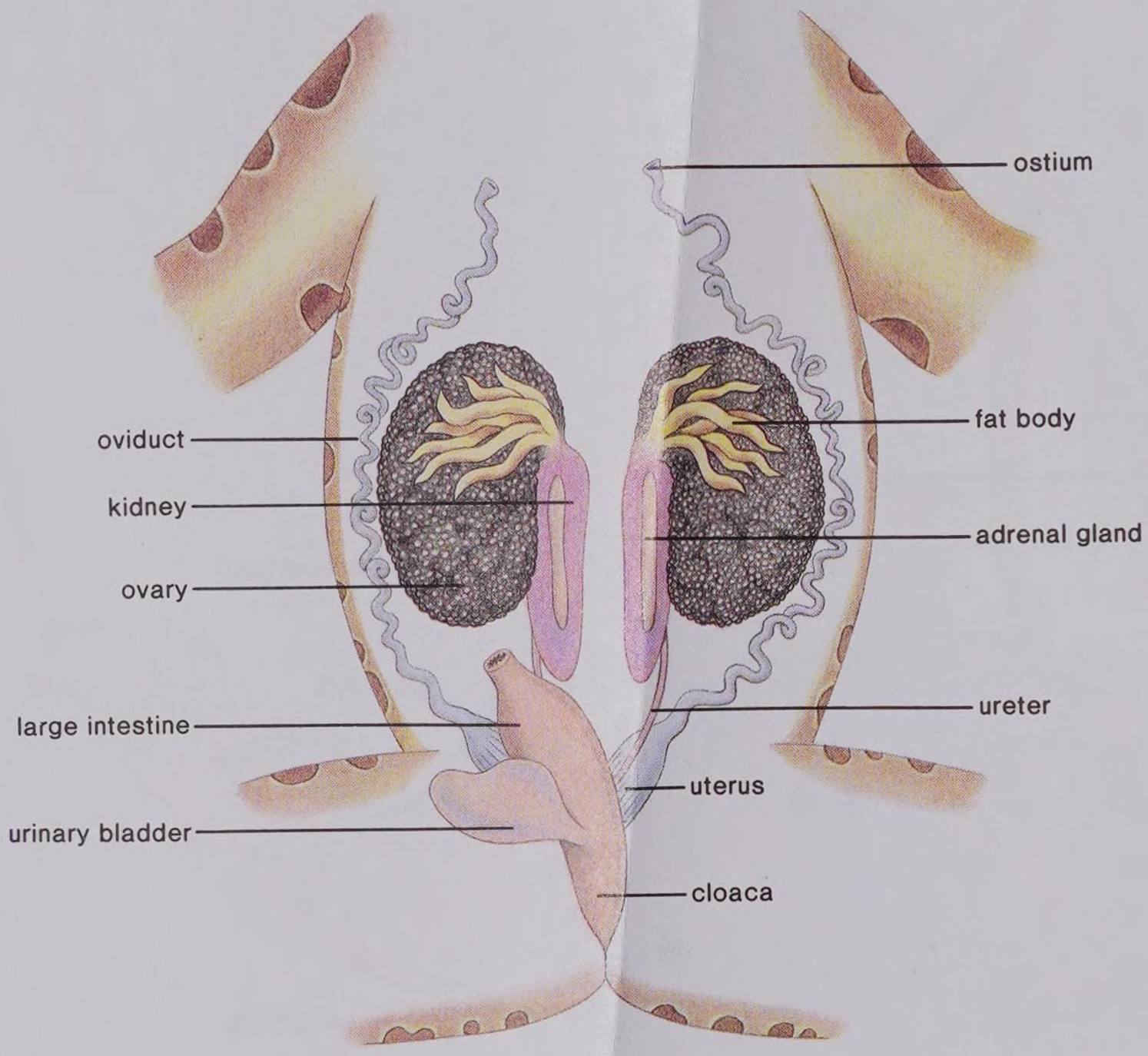
Digestive System

1. Trace the course of the alimentary canal from the *mouth* to the *cloaca.*
2. Identify the parts that are identified in the following. The *esophagus* is a very short connection between the mouth and the stomach. The *liver* has 3 lobes. Between the right and left half of the liver, find the *gallbladder.*
3. Lift the left liver lobe and identify the *stomach,* whitish and J-shaped. The stomach anteriorly connects with the *esophagus* and, posteriorly, with the *small intestine.* You may be able to identify the *pancreas* as a yellowish tissue near the stomach and intestine. At its posterior end, the small intestine connects with the shorter *large intestine.* The large intestine opens into the *cloaca,* which lies beneath the pubic bone. The cloaca is a general receptacle for the intestine, the reproductive system, and the urinary system. It opens by way of the *anus.*

Urogenital System

1. Locate the *kidneys,* which are long, narrow, reddish organs lying against the dorsal body wall.
2. If your animal is a male (fig. 19.6), locate the *testes,* which are yellow, oval organs attached to the anterior portions of the kidneys. From each testis, several small ducts, the *vasa efferentia,* lead to the kidney. These are sperm ducts that carry sperm into the kidney tubules, which also carry urine. The duct leading from the kidney carries both sperm and urine. Yellow *fat bodies* are attached to the testes.

Figure 19.7 Frog, female urogenital system.

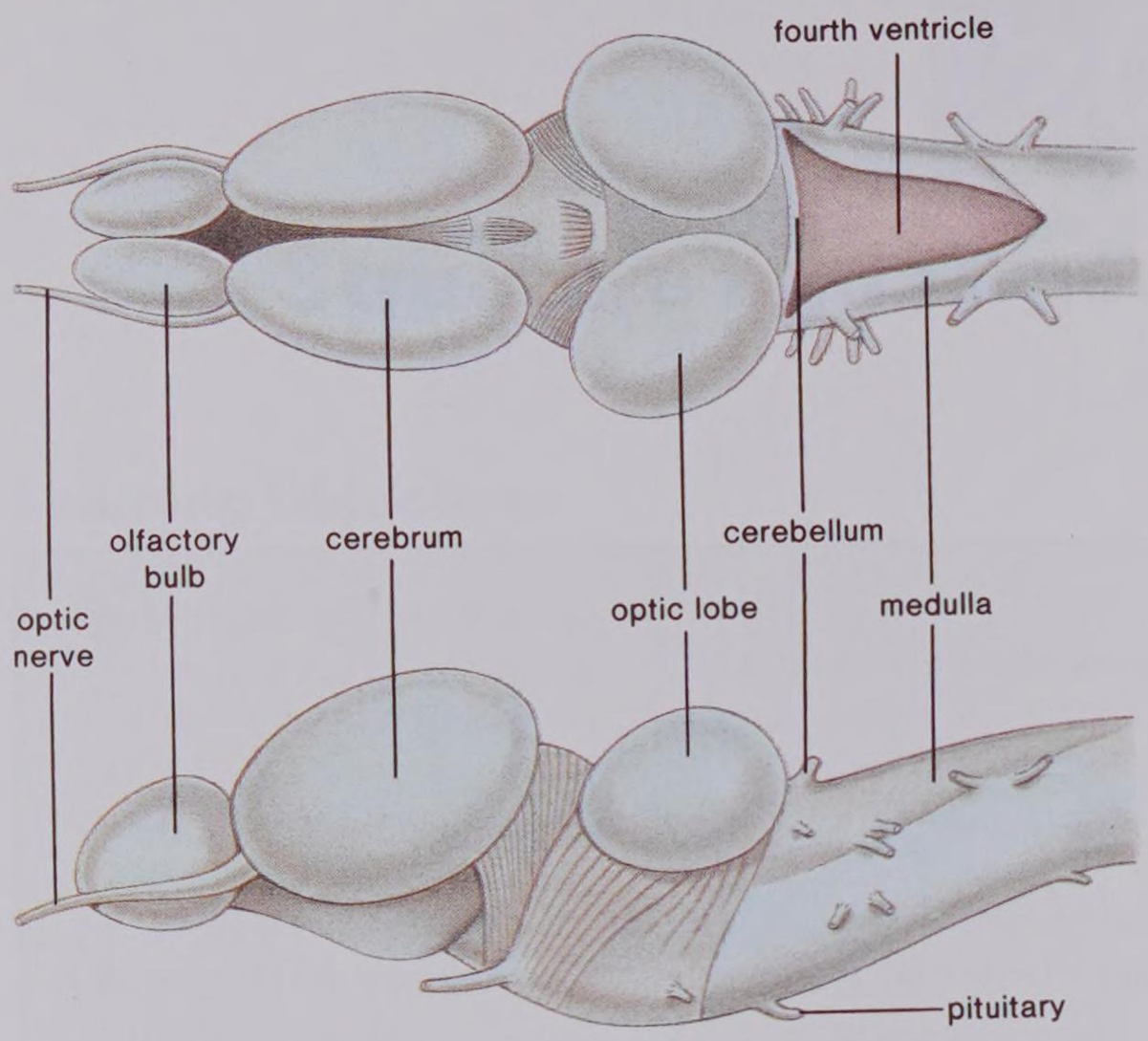


3. If your animal is a female (fig. 19.7), locate the *ovaries,* which are paired and attached to the dorsal body wall.
4. Note the presence of fat bodies attached to the ovaries. Found beneath the ovary is a thick, whitish, highly coiled *oviduct,* which eventually leads to the *cloaca.*
5. Remove the entire left oviduct. Along the posterolateral edge of the *kidney,* which is now visible, find the thin white tube (ureter) that carries urine from the kidney. This also opens into the cloaca.
6. To complete your dissection of the urinary system, split through the bones of the pelvic girdle in the midventral line, carefully separating the bones and muscles until you find the cloaca.
7. Locate the *urinary bladder* attached to the ventral wall of the cloaca. In the frog, urine backs up into the bladder from the cloaca.

Nervous System

1. Carefully remove the skin from the dorsal surface of the head between the eyes and along the *vertebral column.*
2. With your scalpel, carefully shave thin sections of bone from the skull until you expose the *brain.*
3. Then carefully pick away additional small pieces of bone with your forceps until the brain is completely exposed.
4. Continue this same procedure to expose the vertebral column and the *spinal cord,* but leave the nervous system in place. With the help of figure 19.8, identify major portions of the brain.
5. Observe now the spinal cord. Ten pairs of *spinal nerves* are attached to the spinal cord. Each nerve has 2 roots, the dorsal root and the ventral root.
6. Find the *brachial plexus,* where several of the spinal nerves lead to the forelimbs, and the *sciatic plexus,* where several spinal nerves lead to the hind limbs.
7. Trace the large *sciatic nerve* and its principal branches in the hind limb.

Figure 19.8 Frog brain, dorsal and lateral views.



Frog Life Cycle

Study a biomount illustrating the life cycle of a frog and answer the following questions:

Do frogs reproduce in the water or on land? _____

Is a tadpole adapted to water or a land environment? _____

Following metamorphosis, is the adult frog still tied to the water? _____

In what ways? _____

_____

Laboratory Review 19

_____ 1. What type of body system is found only in the echinoderms?

_____ 2. What type of nerve cord is found in lancelets?

_____ 3. Vertebrates are in what phylum?

_____ 4. Does a frog have an open or closed circulatory system?

_____ 5. How many ventricles are found in a frog heart?

_____ 6. Is the digestive system in the frog complete or incomplete?

7. Describe the skeleton of a sea star. Is this different from the skeleton of arthropods?

8. In what respects can the amphibians be considered the "bryophytes of the animal kingdom"?

Laboratory

20

Structure and Organization of Plants

Learning Objectives

Students should be able to

1. identify the external anatomical features of a flowering plant;
2. label slides and models of the major organs of a plant;
3. label the tissues within the organs and state a function for each;
4. identify the cells mentioned and state a function for each;
5. name the zones of a root tip;
6. explain the anatomical difference between monocots and dicots;
7. explain the anatomical and physiological differences between C_3 and C_4 plants.

Introduction

The vegetative organs of a *flowering plant* consist of the roots, stems, and leaves (fig. 20.1). The roots form the *root system,* and the stems and leaves make up the *shoot system.* The root tip and shoot tip contain *meristem tissue* that continually divides and produces new cells. These cells become one of the 3 specialized tissue systems in plants, either dermal, ground, or vascular tissue.

Dermal tissue contains the epidermal cells that make up the epidermis, the outer covering of nonwoody plants. In each part of the plant, the *epidermis* is specialized for a particular function (table 20.1).

Ground tissue forms the bulk of the plants. The cells found in ground tissue may be cells that photosynthesize, that store the products of photosynthesis, or that merely lend support. Support cells are typically hollow, nonliving cells, with thick, strong walls.

Vascular tissue consists of xylem and phloem. Vascular tissue is considered in more detail in the next laboratory. In the meantime, we will mention that *xylem* contains hollow cells (vessel cells and tracheids) that transport water and minerals, and *phloem* contains sieve-tube elements that transport organic nutrients. Sieve-tube elements contain cytoplasm but no nucleus; however, each has a companion cell that does contain a nucleus.

Monocot and Dicot

Flowering plants are divided into 2 groups that differ in regard to the characteristics outlined in figure 20.2. *Monocots* (class Monocotyledonae) have only one cotyledon in the seed, whereas *dicots* (class Dicotyledonae) have 2 cotyledons in the seed. *Cotyledons* are called the seed leaves and serve as a source of food for the developing embryonic plant in the seed. We will discuss 2 other differences between monocots and dicots in this laboratory; these differences involve the arrangement of tissues in the stem and leaves. Seed structure was discussed in detail in Laboratory 16, and the number of flower parts is discussed in Laboratory 22.

Figure 20.1 The root and shoot systems of a land plant.

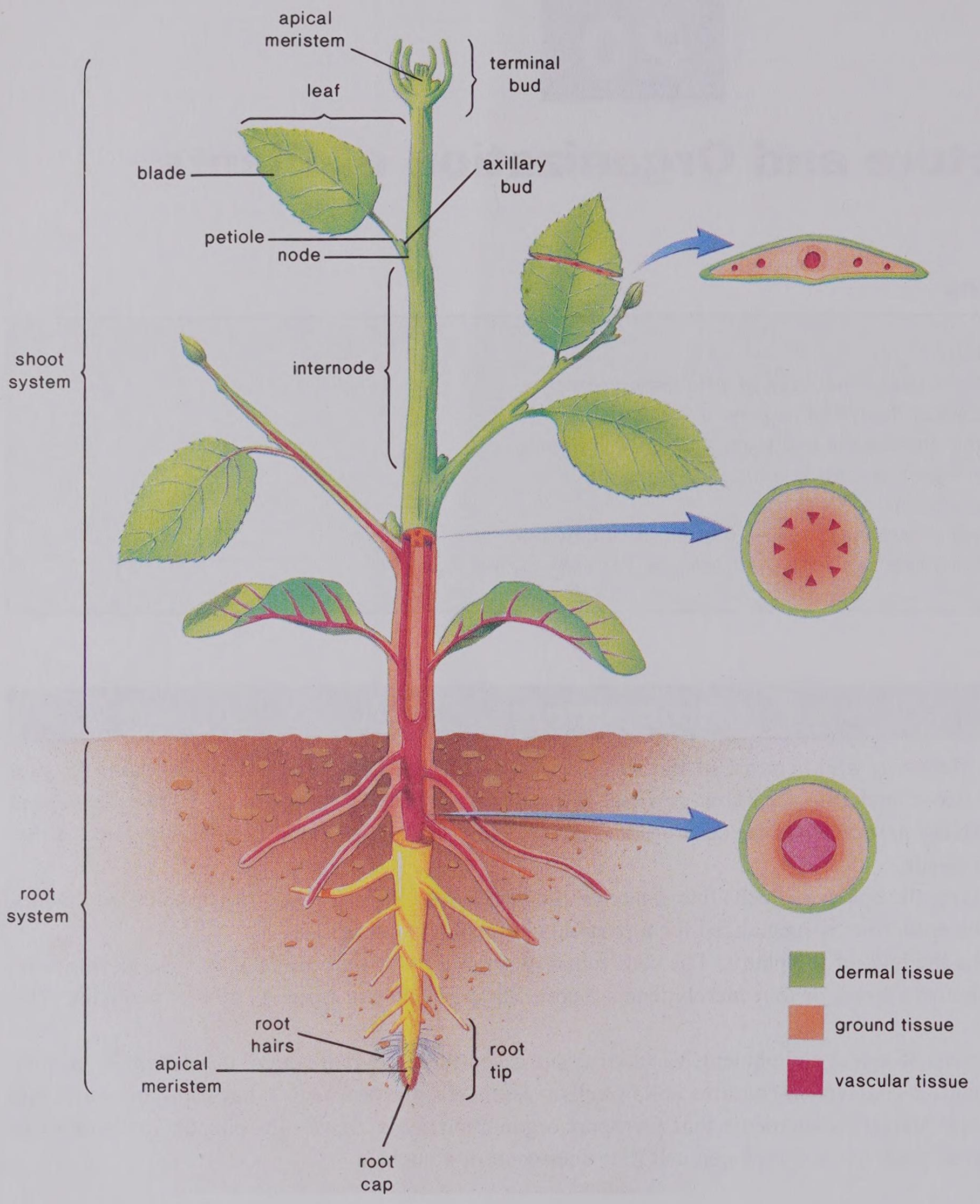
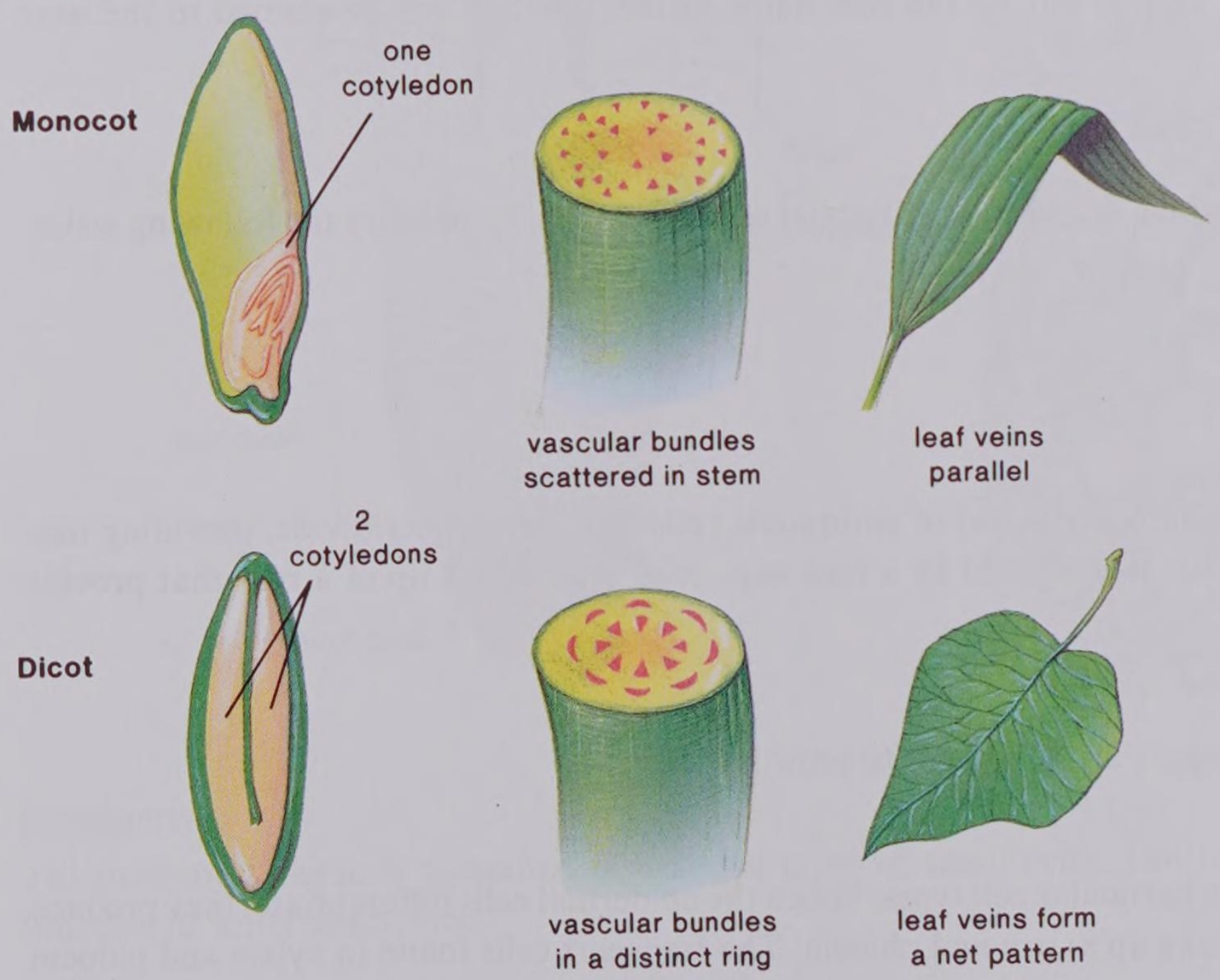


Table 20.1 Vegetative Organs and Major Tissues

	Root	Stems	Leaves
Function	Absorb water and minerals Anchor plant and store materials	Transport water and nutrients Support leaves, etc., and help store materials	Carry on photosynthesis
Tissue			
Epidermis (dermal tissue)	Root hairs absorb water and minerals	Protect inner tissues	Cuticle-H_2O loss protection Stomata carry on gas exchange
Cortex (ground tissue)	Store products of photosynthesis	Carry on photosynthesis, if green	(not present)
Xylem and phloem (vascular tissue)	Transport water and nutrients	Transport water and nutrients	Transport water and nutrients
Pith (ground tissue)	Store products of photosynthesis	Store products of photosynthesis	(not present)
Mesophyll (ground tissue)	(not present)	(not present)	
Spongy layer Palisade layer			Gas exchange/Photosynthesis Photosynthesis

Figure 20.2 Monocot versus dicot plants.



External Anatomy

S 1. Remove a *bean* (dicot) seedling from the container in which it is growing.
S 2. With the help of figure 20.1, identify the *root system* and the *shoot system.* Note that the seedling has a *primary root* and many *lateral roots.*
S 3. In the shoot system, identify the *stem* and the *leaves.* At the tip of the stem find the *terminal bud,* where meristem tissue produces new cells that cause an increase in length of the stem.
S 4. In the stem, note the *nodes* to which the leaves are attached and the *internodes,* or portion of the stem between the nodes. It's possible that, if your plant is very young, you will see 2 fleshy lobes attached to the stem. These are remnants of the *cotyledons.*
S 5. Note the leaf with a *blade* (expanded portion of a leaf) and the *petiole* (stalk of a leaf). Look in the notch between petiole and stem for an *axillary bud.* These, if active, produce side branches of plants.

With the help of figure 20.2, give as much evidence as you can that the bean seedling is a dicot plant. Evidence:

1. _____

2. _____

Root System

Roots are underground structures that anchor the plant, absorb water and minerals, and store the products of photosynthesis. The absorption function is mainly carried out by the root hairs, structures that will be studied in the next laboratory.

Dicot Root Tip

S Examine the model and slide of a root tip, which display a longitudinal section (fig. 20.3). Identify the following zones.

1. zone of cell division
2. zone of elongation
3. zone of maturation

Zone of Cell Division

Meristem tissue is found in this zone. This tissue is composed of embryonic cells that continually divide, providing new cells for continuous growth. The meristem tissue is protected by a root cap, dead cells at the tip of a root that provide protection as the root grows.

Zone of Elongation

In this zone, rows of newly produced cells elongate as they begin to grow larger.

Zone of Maturation

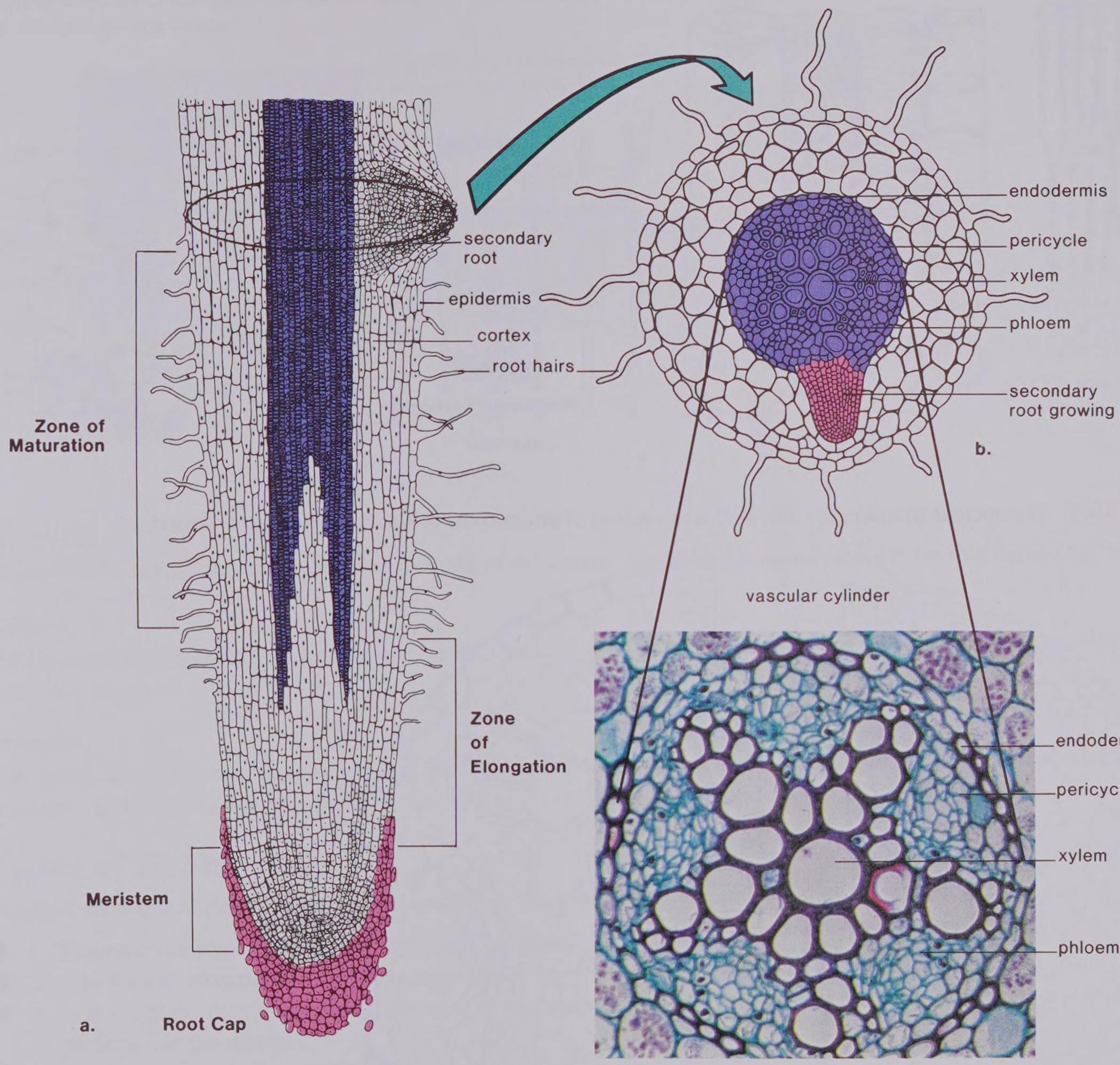
In this zone, the cells become differentiated into particular cell types. When the epidermal cells differentiate, they produce root hairs. Also noticeable are the cells that make up xylem and phloem. The transport cells found in xylem and phloem are shown in figure 20.4.

The *primary growth* of a root occurs in the manner we have just described: meristem tissue produces new cells that elongate and become mature. Secondary growth of roots does occur, but it occurs similar to that in stems. We will restrict our discussion of secondary growth to stems.

Dicot Root (Cross Section)

S Examine a slide of a cross section of the mature buttercup (*Ranunculus*) *root* (fig. 20.5). Use both low and high power and identify the following tissues.

Figure 20.3 ***a.*** Longitudinal section of root tip showing the major zones. ***b.*** Cross section of root tip. ***c.*** Photomicrograph of vascular cylinder.



Epidermis

The outermost tissue is composed of a single layer of small cells. The root hairs are extensions of these cells, which function in water absorption.

Cortex

Composed of *parenchyma cells,* the cortex constitutes a large portion of the root. This tissue functions in storage; note the many stained starch grains in the cells.

Endodermis

Thick-walled cells mark the boundary of the *vascular cylinder,* the central portion of the root. The endodermal cells are bordered on the 4 sides that contact other endodermal cells, but not the 2 sides that contact the cortex and pericycle, by a strip of waxy material known as the *Casparian strip.* This strip will not permit water and solutes to pass between adjacent cells; therefore, the only access to the vascular cylinder is through the living endodermal cells (fig. 20.6). These cells are essential in regulating absorption by a plant.

Figure 20.4 Representative cells of vascular tissue. ***a.*** Xylem includes tracheids and vessel elements. ***b.*** Phloem includes sieve-tube cells and companion cells.

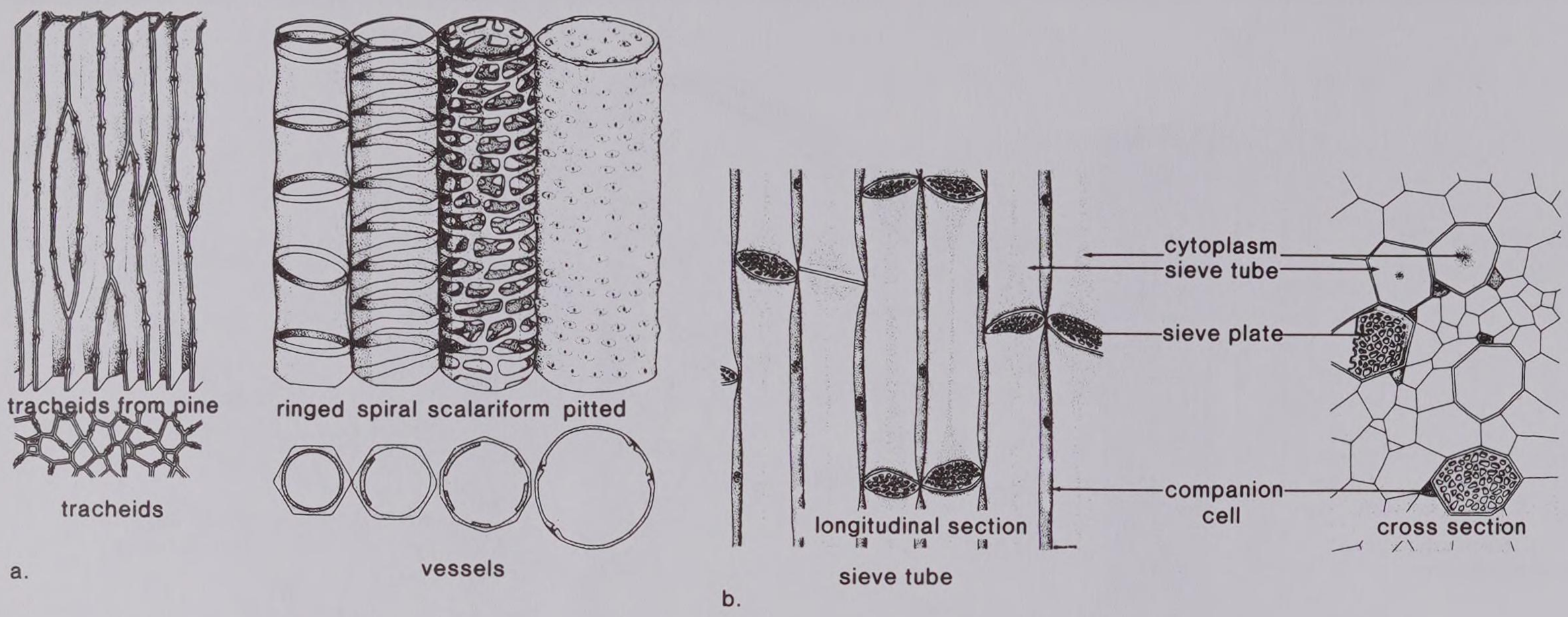


Figure 20.5 *Ranunculus* (buttercup) root. ***a.*** Cross section of entire root. ***b.*** Vascular cylinder enlarged.

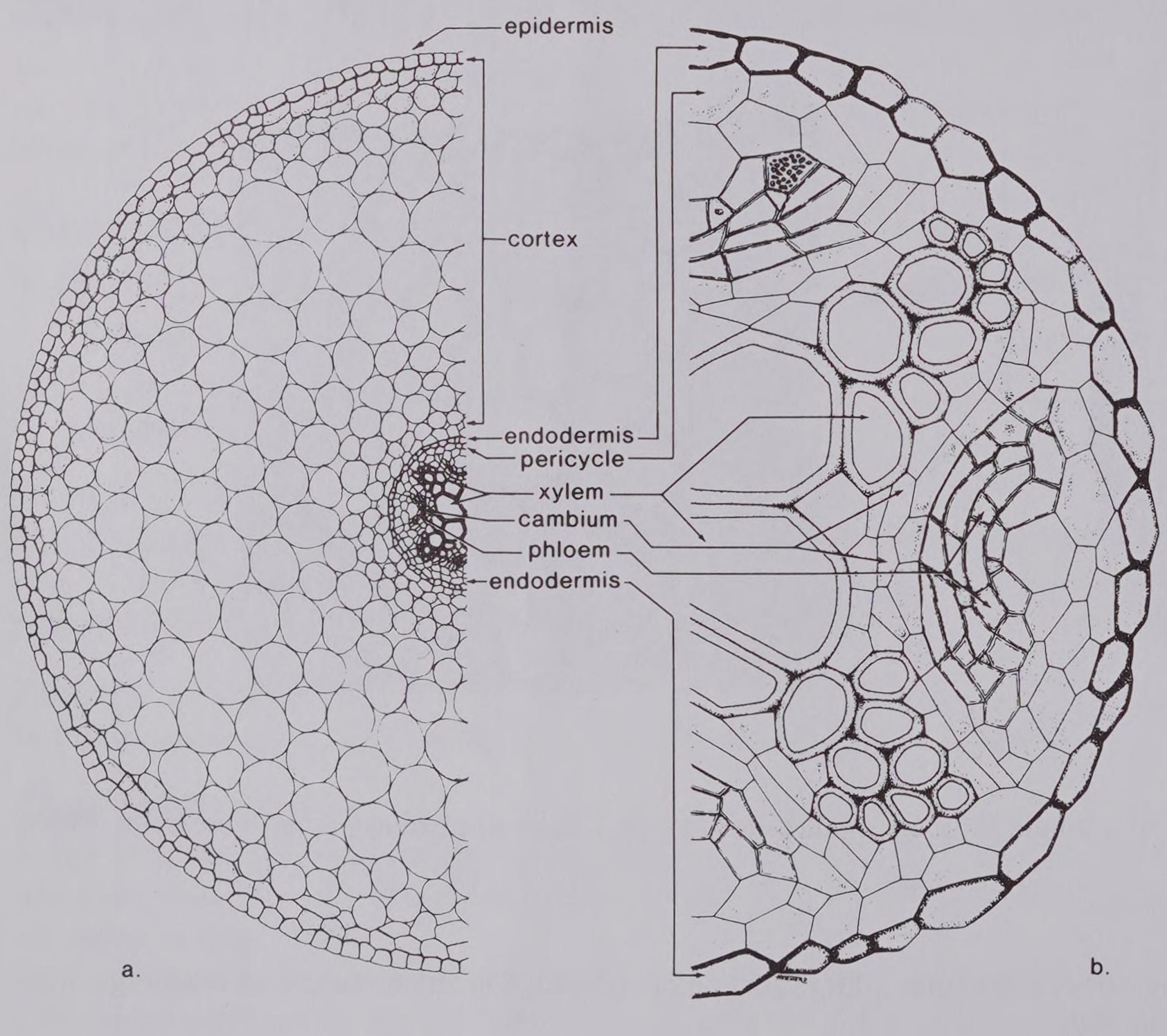
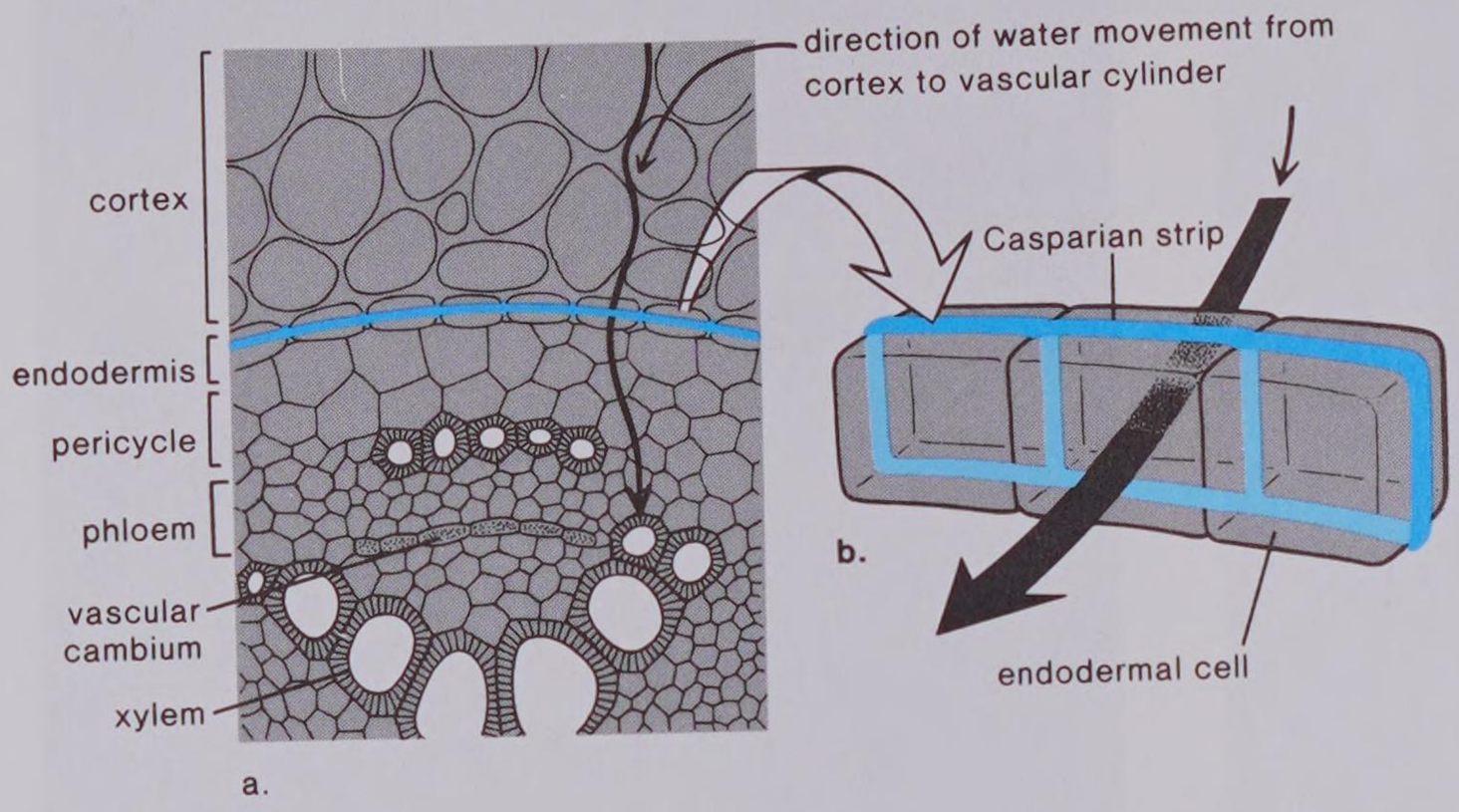


Figure 20.6 Movement of water and solutes from cortex to vascular cylinder by way of endodermal cells. ***a.*** Diagram illustrating relationship of root tissues and position of Casparian strip (*colored line*). ***b.*** Endodermal cells in detail, also showing location of Casparian strip. Because of the Casparian strip, water and solutes must pass through the cytoplasm of endodermal cells (*arrow*).



Pericycle

Pericycle cells are similar in appearance to those of the cortex. Secondary roots originate from this tissue (fig. 20.6*b*).

Xylem

The large vessel cells form a star shape with several "arms." This tissue functions in the conduction of water and minerals from roots to leaves.

Phloem

This tissue is located between the arms of the xylem and is composed of *sieve-tube elements* and *companion cells*. It functions in the conduction of organic molecules (food) from leaves to roots.

Types of Roots

Roots develop in a variety of patterns depending on the type of plants on which they form.

1. Examine the root systems on display and use figure 20.7.
2. Use your observations to complete table 20.2.
3. List the plant (common *name*), the *type* of root system, and the *special functions* that this type of root system perform for the plant.
4. Use your text or other references.

Figure 20.7 Three types of roots. ***a.*** A taproot may have secondary roots in addition to a main root. ***b.*** A fibrous root has many secondary roots with no main root. ***c.*** Adventitious roots, such as prop roots, extend from the stem.

a.

b.

c.

Table 20.2 Plants and Their Root Systems

Plant	Type of Root	Specialized Function

Shoot System

The shoot system consists of the *stems* and *leaves*. *Herbaceous* stems are nonwoody stems. Usually monocots remain herbaceous throughout their entire lives; some dicots such as those that live a single season are also herbaceous. Other dicots such as trees become woody as they mature.

Primary Growth of Stems

Primary growth of stems arises as the meristem in the terminal bud divides and produces new cells. The exact same zones that we observed in the root tip can also be identified in the shoot tip.

Dicot Herbaceous Stem

Ⓢ Examine a slide of a cross section of a *herbaceous dicot stem* of buttercup (*Ranunculus*) (fig. 20.8) and note these tissues:

Epidermis: protective; can carry on gas exchange and photosynthesis.
Cortex: may carry on photosynthesis or store nutrients.
Vascular bundle: contains both xylem and phloem tissues. In a dicot stem the vascular bundles occur in a ring.
Pith: stores the products of photosynthesis.

Monocot Herbaceous Stem

Ⓢ Examine a slide of a cross section of a *herbaceous monocot stem* of corn *stem* (*Zea*) (fig. 20.9). In monocots the vascular bundles are said to be scattered. Why? _____

Types of Stems

Most plants simply have erect stems, but many have stems that perform specialized functions and are modified accordingly. Ⓢ Examine figure 20.10 and the stems that may be on display. Use your text and other references to complete table 20.3. List the plant (common name), the type of *modification,* and the *special function* that this modification performs for the plant.

Figure 20.8 *Helianthus* (sunflower) stem, a herbaceous stem, in cross section.

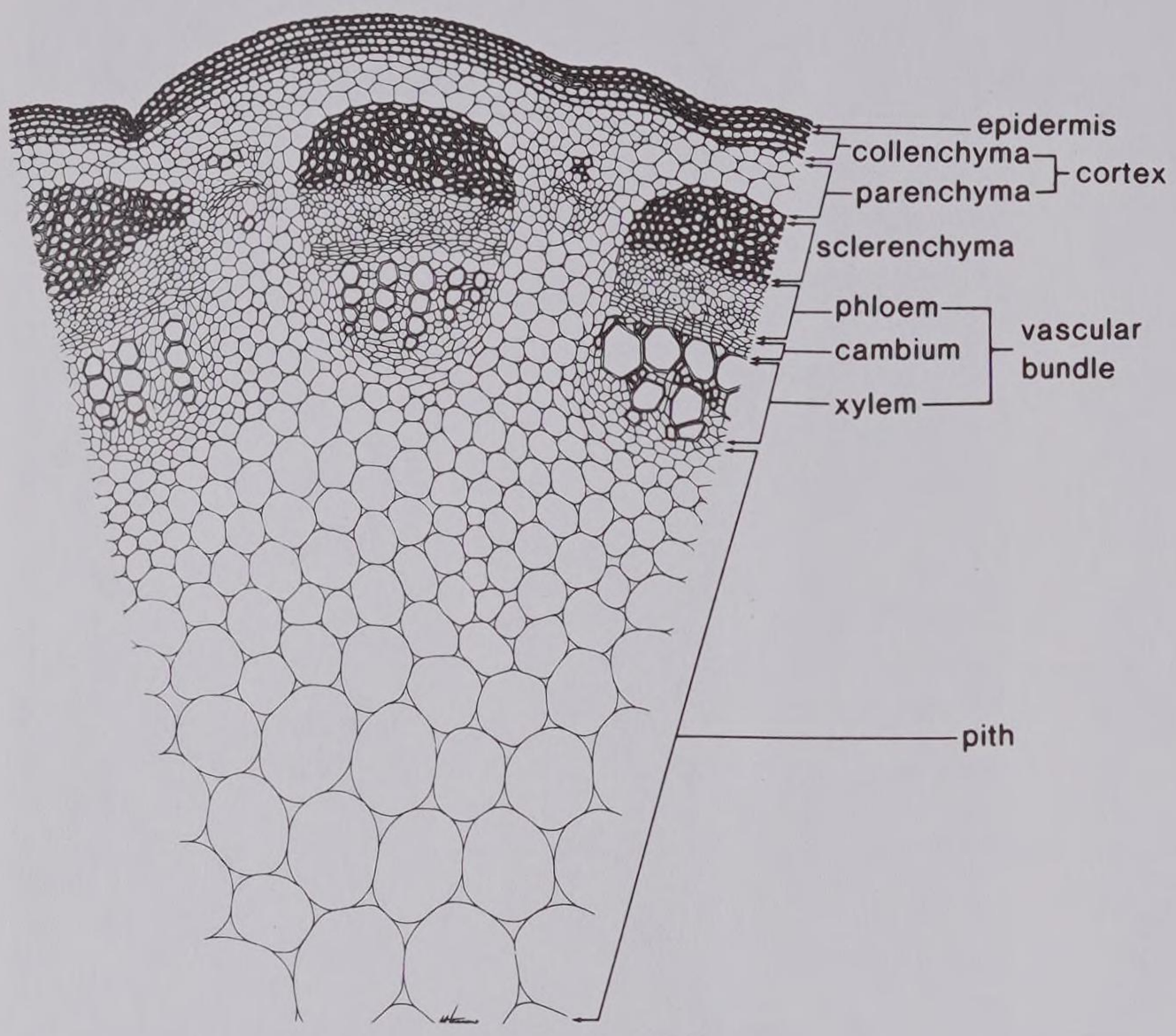
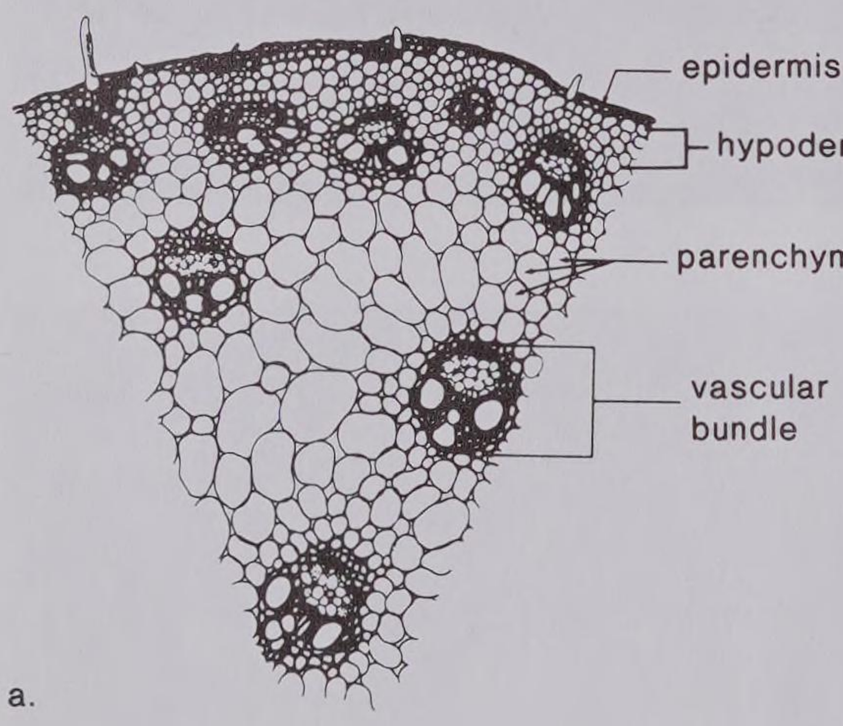
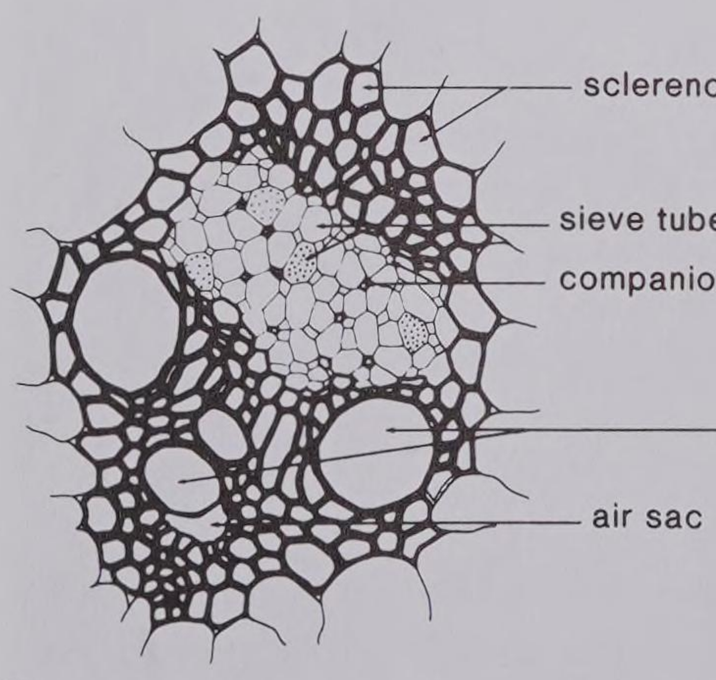


Figure 20.9 Cross section of a corn (monocot) stem. ***a.*** Vascular bundles. ***b.*** Enlargement of vascular bundle. ***c.*** Photomicrograph of vascular bundle.



a.



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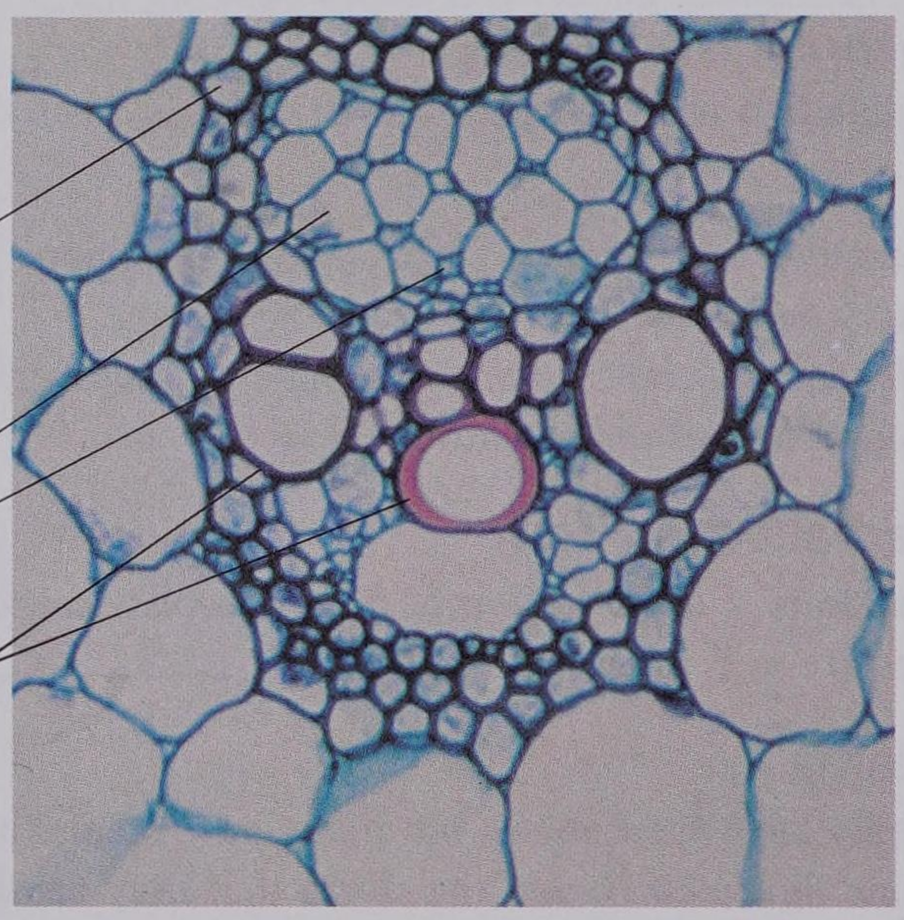
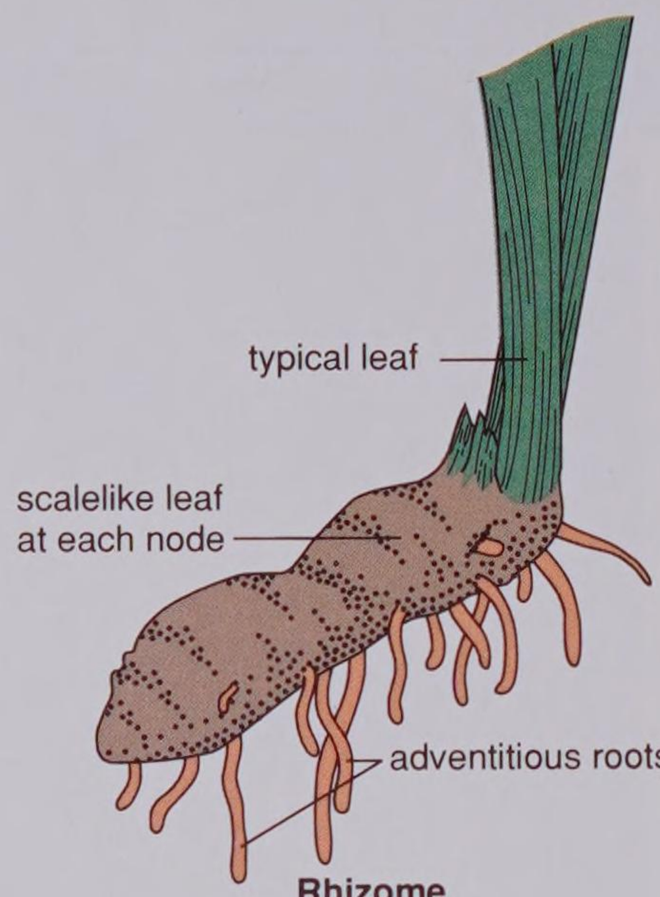
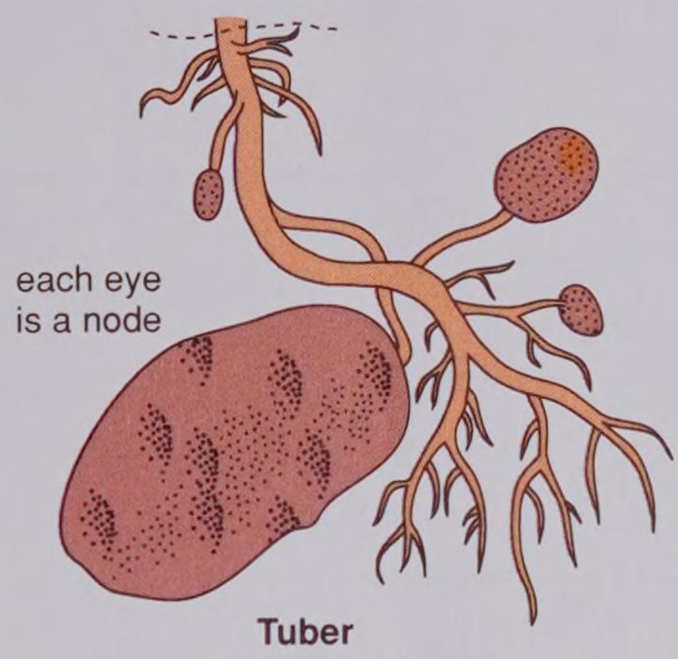


c.

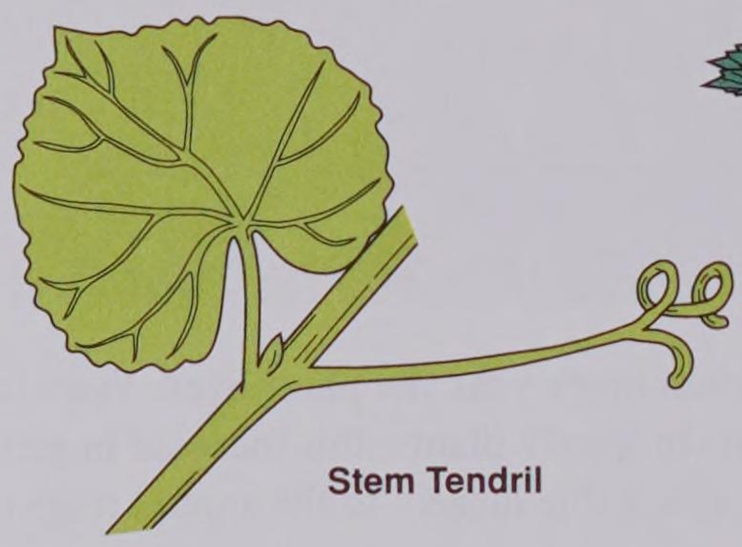
Figure 20.10 Types of specialized stems.



Rhizome

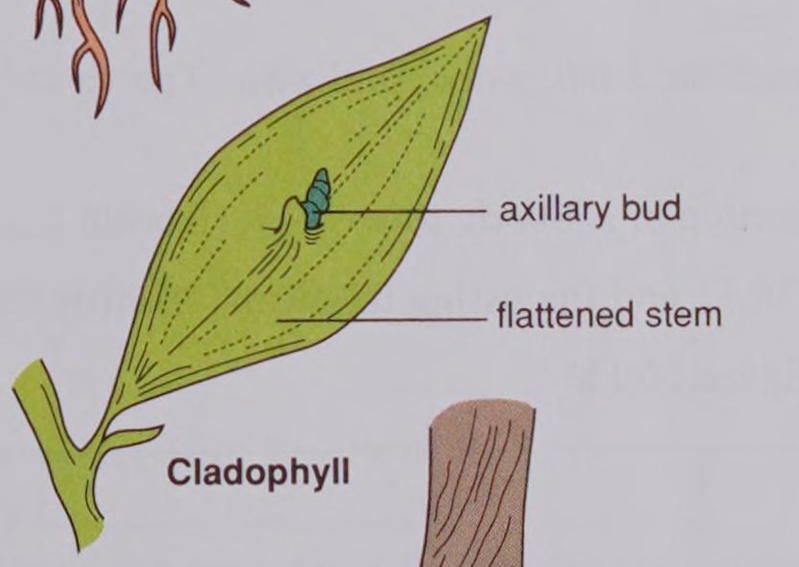


Tuber

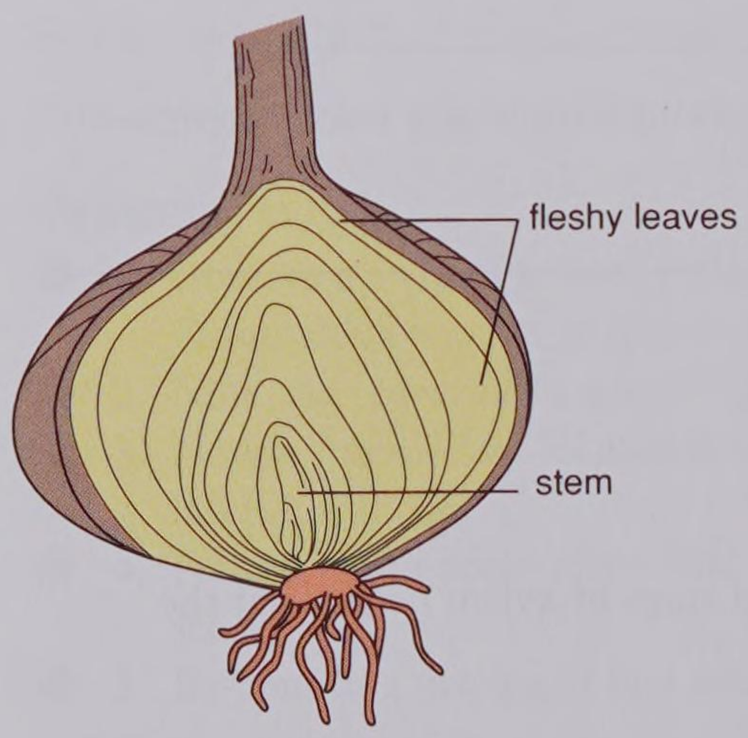


Stem Tendril

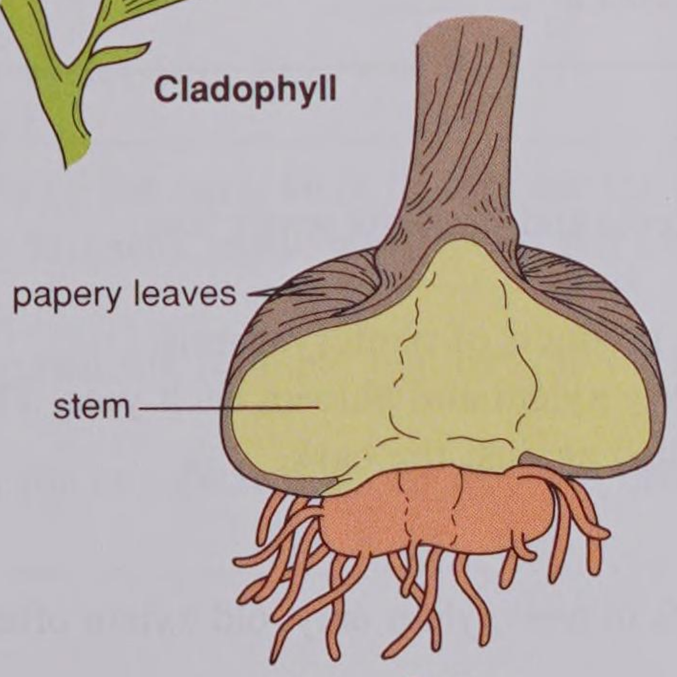
Stolon



Cladophyll



Bulb



Corm

Table 20.3 Modified Stems

Plant	Type of Modification	Function

Secondary Growth: Woody Stem

Dicot stems contain *vascular cambium,* which gives rise to new xylem and phloem every year the plant lives. Vascular cambium is lateral meristem—when it divides it increases the *girth* of the plant. In woody plants, this increase in girth is quite noticeable, as the diameter of the tree trunk gets larger. This increased size is due largely to the annual rings of xylem that build up year after year.

A woody stem has 3 regions: *bark, wood,* and *pith.* The vascular cambium occurs between the bark and the wood.

S 1. View a slide of a cross section of a *woody dicot stem* (labeled _____).

S 2. With the help of figure 20.11 and the listing following, identify the *regions* and *tissues* of a woody stem.

How old is the stem in figure 20.11? _____
How do you know? _____

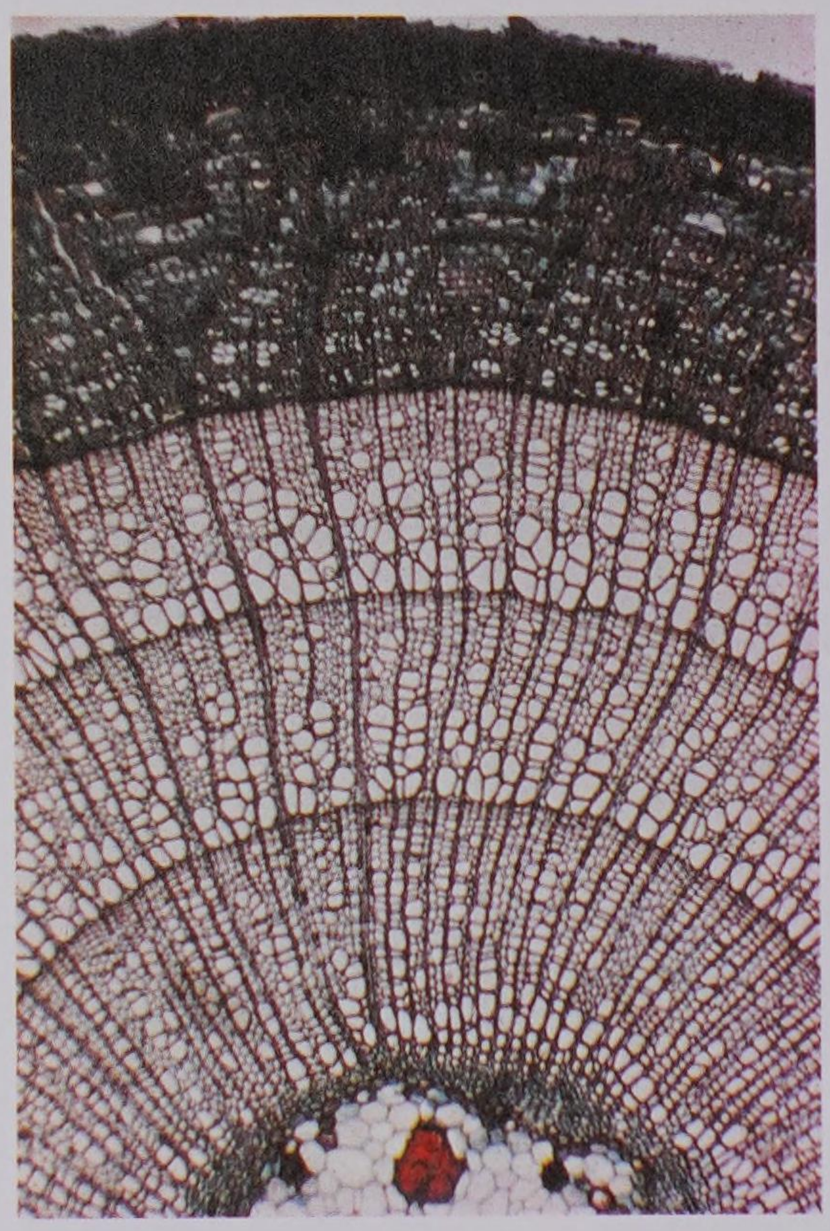
Bark

Cork: replaces epidermal tissue; protects and prevents water loss.
Cortex: stores nutrients and water.
Phloem: transports nutrients and the products of photosynthesis.
Vascular cambium: produces secondary xylem and phloem each year. The annual rings of xylem persist but the previous year's phloem gets crushed against the bark.

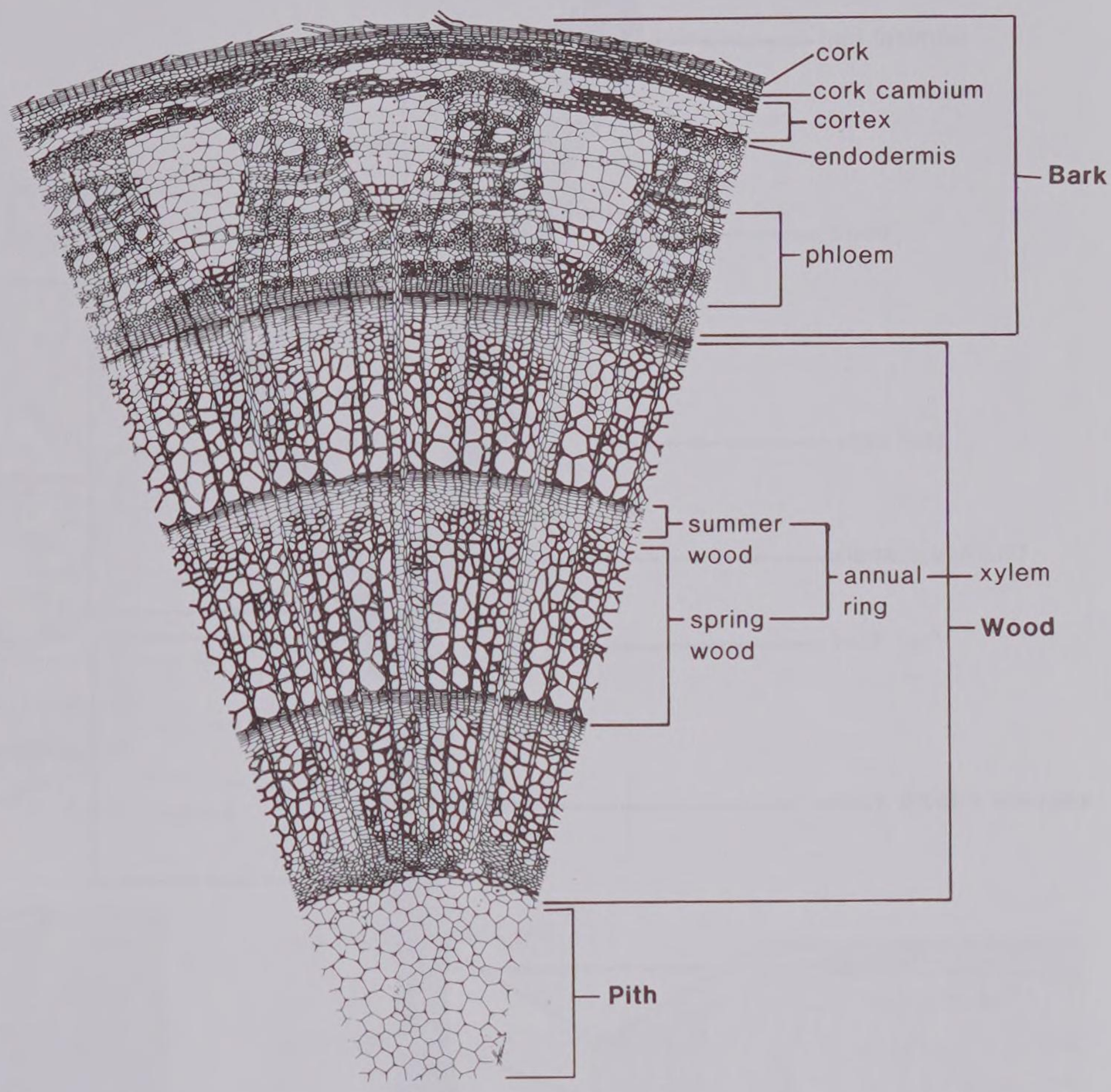
Wood

Xylem: transports water and nutrients in new xylem only; old xylem often present as annual rings.

Figure 20.11 Woody dicot stems after 3 years' growth. ***a.*** Photomicrograph. ***b.*** Drawing indicating location of tissues.



a.



b.

Pith

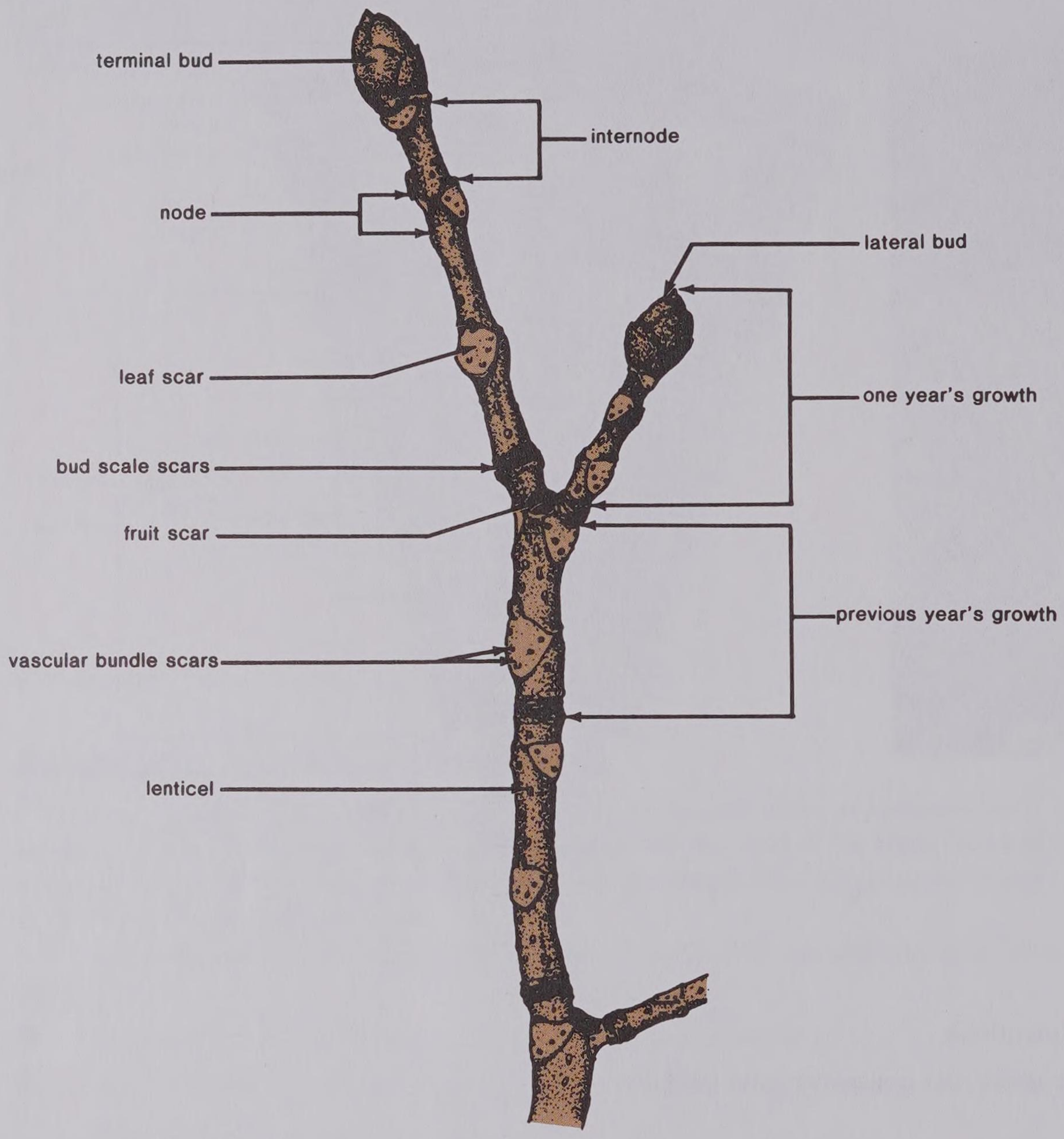
Stores the products of photosynthesis.

Pith or xylem ray: vascular tissue that transports water laterally.

Winter Twig

1. Observe a twig of a woody plant in the winter (fig. 20.12).
   From which species of tree is your twig? _____
2. Note the *terminal bud* located at the tip of the twig. Here growth for the current year will originate.
3. Note a *lateral bud* located just above a leaf scar, made when a leaf fell from the twig. Lateral buds produce lateral branches.
4. Note the *bud scale scars:* bud scales protect the terminal bud but fall off as the new stem develops, leaving scars.
5. By counting groups of bud scale scars, you can determine the age of a branch.
   How old is the twig you are studying? _____

Figure 20.12 External structure of a woody stem.



Leaves

The bulk of all monocots and dicots have *C_3 photosynthesis,* so called because the first detectable metabolite during photosynthesis is a C_3 molecule. However, *C_4 photosynthesis,* so called because the first detectable metabolite during photosynthesis is a C_4 molecule, occurs in nearly half of all *grasses* and in many of our important grain (grass) crops. The C_4 species have very high photosynthetic rates and other physiological characteristics that make them well adapted to hot, arid environments.

C_3 Leaves

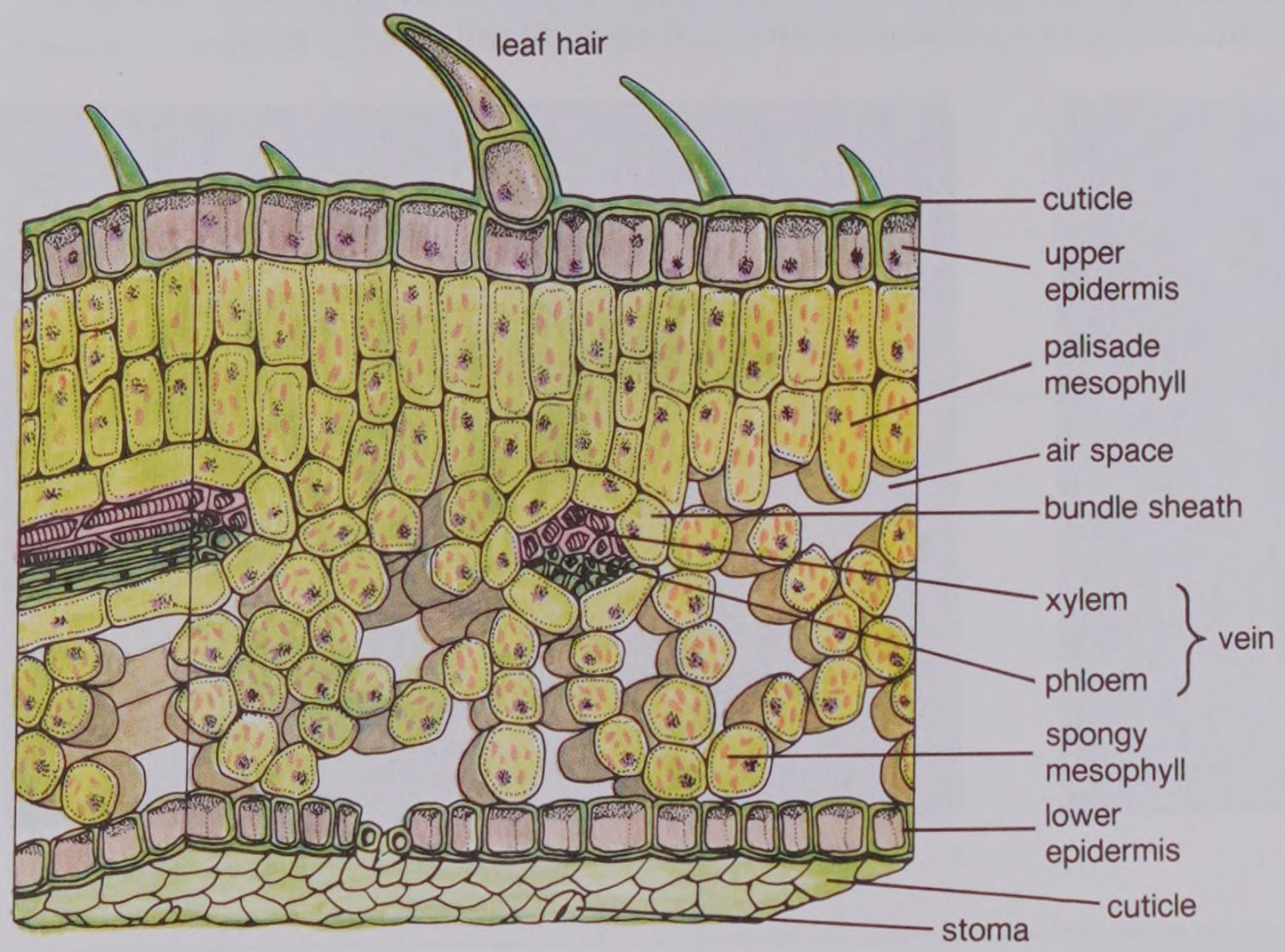
Examine the cross section slide of the available C_3 leaf *Syringa* (Lilac), *Ligustrum* (Privet), or _____, and note the following *tissues* (fig. 20.13*a*).

Epidermis

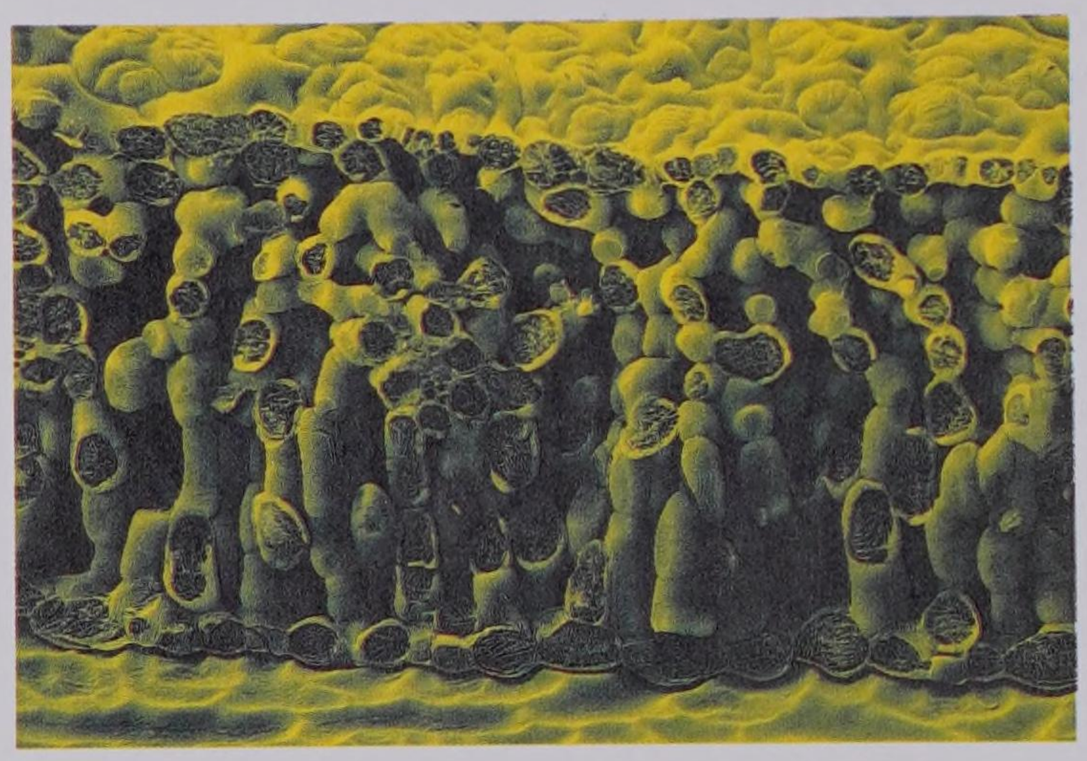
Upper: epidermal cells with waxy cuticle interrupted by a few (or no) *stomata:* pores in the leaf, surrounded by guard cells, that function in gas exchange.

Lower: epidermal cells with waxy cuticle interrupted by many stomata. When the guard cells are turgid, a stoma is open; when the guard cells are flaccid, a stoma is closed.

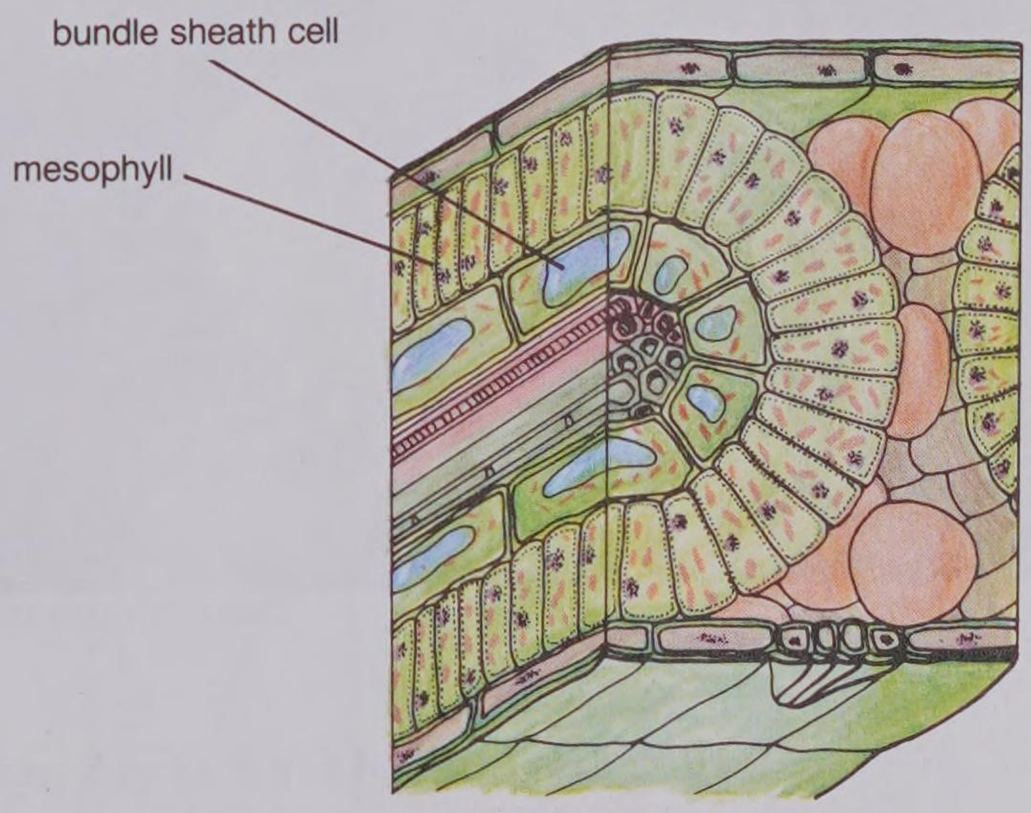
Figure 20.13 Leaf. ***a.*** Drawing of C_3 leaf adapted to temperate climate. ***b.*** Scanning electron micrograph of mesophyll. ***c.*** Drawing of C_4 leaf adapted to hot, dry climate.



a.



b.



c.

Leaf Veins

Leaf veins contain the vascular tissue, *xylem* and *phloem.* In *dicots,* the leaf veins form a *net* pattern; in *monocots,* the leaf veins are *parallel.*

Mesophyll

Palisade layer: parenchyma cells with chloroplasts. These cells are responsible for most of the photosynthesis carried on by the plant.

Spongy layer: parenchyma cells with air spaces and chlorophyll. Oxygen and carbon dioxide diffuse across the plasma membrane of these cells.

C_4 Leaves

S 1. Examine the cross section slide of the leaf of a C_4 plant such as sorghum, corn, sugar cane, or _____.

S 2. Note the striking difference in the appearance of the vein (fig. 20.13*c*). In C_4 plants, the cells in the surrounding *sheath* are large and often contain many organelles, which are located near the side of the cell

Figure 20.14 Leaf modifications. ***a.*** The spines of a cactus plant are leaves modified to protect the fleshy stem from animal consumption. ***b.*** The tendrils of a cucumber are leaves modified to attach the plant to a physical support. ***c.*** The Venus's-flytrap has leaves that are modified to serve as a trap for insect prey. When triggered by an insect, the leaf snaps shut. Once shut, the leaf secretes digestive juices that break down the soft parts of the insect's body.

a.

b.

c.

closest to the vascular bundle. This gives the bundle a wreathlike appearance. These cells carry on photosynthesis when the mesophyll cells pass them CO_2. It is the ability of these cells to pick up CO_2 efficiently that allows these leaves to continue to photosynthesize under the limiting conditions of hot, arid environments.

Types of Leaves

Some plants have leaves that are highly specialized to perform adaptive functions for the plant.

Ⓢ Examine the plants on display (fig. 20.14) and complete table 20.4, using your observations.

Table 20.4 Leaf Modifications

Plant	Modification	Function

Laboratory Review 20

_____ 1. What are the storage tissues of roots and stems?
_____ What do they store?

_____ 2. In the temperate zone, how do you determine the age of a woody stem?

_____ 3. What is the function of stomata?

_____ 4. In what layer of a leaf are the chloroplasts concentrated?

_____ 5. What tissue is responsible for the transportation of water from the root to the leaf?

6. What are the 3 types of organs found in plants and the functions of each?

7. What is the function of the meristematic tissues? Where can they be found in plants such as trees?

8. How does a C_4 plant differ from a C_3 plant? Name one of each kind.

9. What are the scattered structures in monocot stems? What tissues do they contain? What is the function of these tissues?

Reference

Snow, B. L., ed. *Biological Laboratory Exercises*. Boston, MA: Suffolk University.

Laboratory

21

Water Absorption and Transport in Plants

Learning Objectives

Students should be able to

1. state the primary force that causes water to rise in vascular tissue;
2. identify vascular tissue in roots, stems, and leaves;
3. identify stomata in leaves and relate their operation to the rise of water in xylem;
4. estimate, in general terms, the volume of water transpired by a plant, the number of stomata in a leaf, and the quantity of root hairs in a root;
5. predict the net movement of water into or out of root hairs according to the tonicity of groundwater.

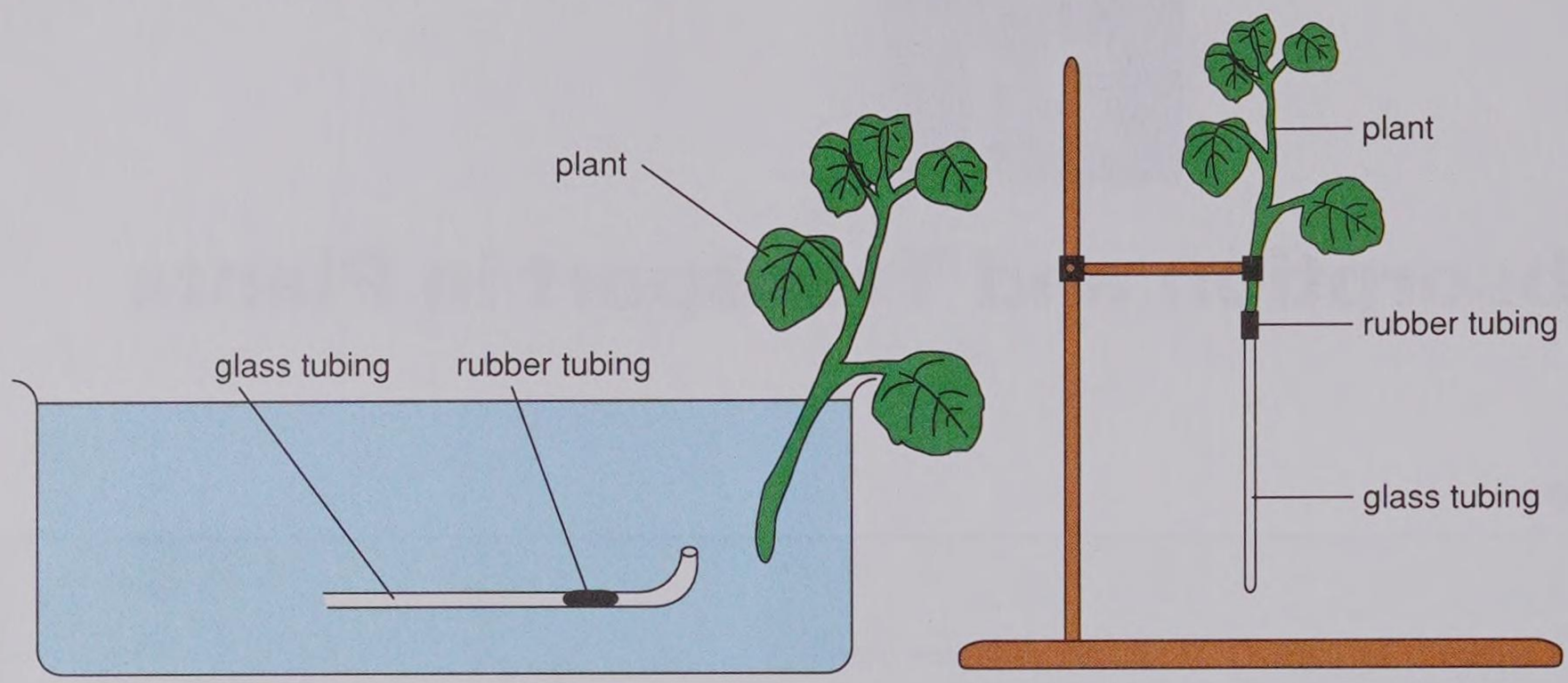
Introduction

Water loss through the leaves of land plants is reduced by the presence of a waxy *cuticle* on the exterior surface of the epidermis. The control of water loss from the plant is accomplished by the *stomata,* pores whose size is regulated by *guard cells*. Originally, this water is absorbed by *root hairs;* after absorption it enters the vascular tissue, *xylem,* and then is conducted up the stem to the *leaves*. The study of plant physiology has revealed that the evaporation of water at the leaf, termed *transpiration,* creates a force that pulls on the column of water in xylem so that the water rises from the roots to the leaves.

Transpiration

S 1. Assemble a transpirometer as follows (fig. 21.1).
S 2. First, fit half of a 4 cm piece of *rubber tubing* tightly over one end of a 30 cm long *glass tube.*
S 3. Immerse the assembled glass and rubber tube in a tub of *water* so that it will fill.
S 4. Check that there are *no air* bubbles in the tube.
S 5. Cut off a portion of a geranium plant (stem and leaves).
S 6. Place the cut end of this stem into a large tub of *water,* but keep the leaves dry.
S 7. With a sharp razor blade, held under water to prevent drying or sealing of the vascular system, *cut* off another 1.5 cm of the stem, cutting at an oblique angle.
S 8. Underwater, *fit* the cut stem end of the seedling tightly into the rubber tubing that has been filled with water by submerging it in the same tub and squeezing out all of the air bubbles.
S 9. Keep the leaves as *dry* as you can.
S 10. If necessary, wind a rubber band, or tie a string tightly, around the juncture for added tightness (do not crush the stem). *Remove* the assembled apparatus from the water.
S 11. Hold the whole assembly so that the stem cutting is *upright.*
S 12. *Mount* the assembly on a rod of a ring stand. The water should not run out due to the adhesive and cohesive properties of water and the formation of a vacuum.
I 13. With a wax pencil, *mark* the water level at the lower end of the transpirometer.
I 14. After *one hour* or so, record in the space provided the distance, in millimeters, between the initial and terminal water marks.

Figure 21.1 Transpiration experiment.



15. Calculate the amount of water transpired and the rate of transpiration in terms of cubic centimeters per hour.

Time 1 _____

Time 2 _____

Total time _____ min. = _____ hrs. (min/60)

Radius of tubing (r) _____ mm

Distance water moved (h) _____ mm

Amount of water transpired _____ mm^3 (πr^2h) = _____ cm^3 ($mm^3/1000$)

Rate of transpiration _____ cm^3/hr.

Table 21.1 estimates the amount of water that is lost by transpiration from various plants per day and per growing season. A researcher counted the number of leaves on a 47-foot maple tree and found that there were 177,000 leaves. In general terms, how much surface area does your cutting have compared to the entire plant above (count the number of leaves on your cutting and divide by 177,000 leaves, multiply this by 100 to give the percent of a full tree that this cutting represents). _____

Would this account for the difference in your data and those given in table 21.1? _____

Table 21.1 Transpiration Rates (approximate)

Per Day Midsummer		
Ragweed		5–6 liters (5,000–6,000 cm^3)
10-ft apple tree		10–20 liters (10,000–20,000 cm^3)
12-ft cactus		.02 liter (20 cm^3)
Coconut palm		70–80 liters (70,000–80,000 cm^3)
Date palm		400–500 liters (400,000–500,000 cm^3)
Per Growing Season		
Tomato	100 days	115 liters (115,000 cm^3)
Sunflower	90 days	480 liters (480,000 cm^3)
Apple tree	188 days	6,800 liters (6.8×10^6 cm^3)
Coconut	365 days	15,900 liters (1.59×10^7 cm^3)
Date palm	365 days	132,000 liters (1.32×10^8 cm^3)

Modified from *Botany: A Human Concern* by D. Rayle and L. Wedberg. Copyright © 1975 W. B. Saunders, Philadelphia, PA.

Figure 21.2 ***a.*** Surface view of epidermis and guard cells. ***b.*** Photomicrograph of guard cells showing that they contain chloroplasts.

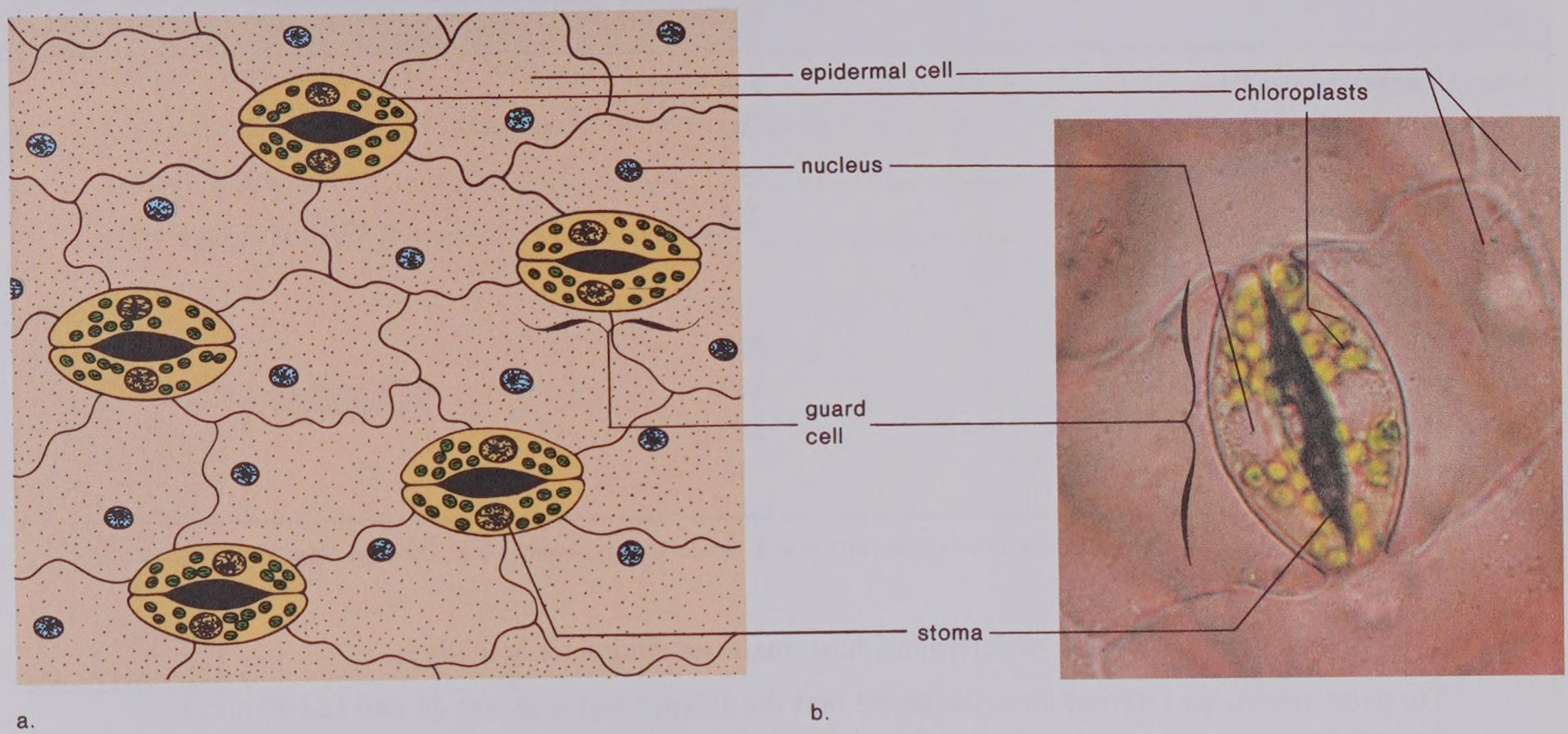
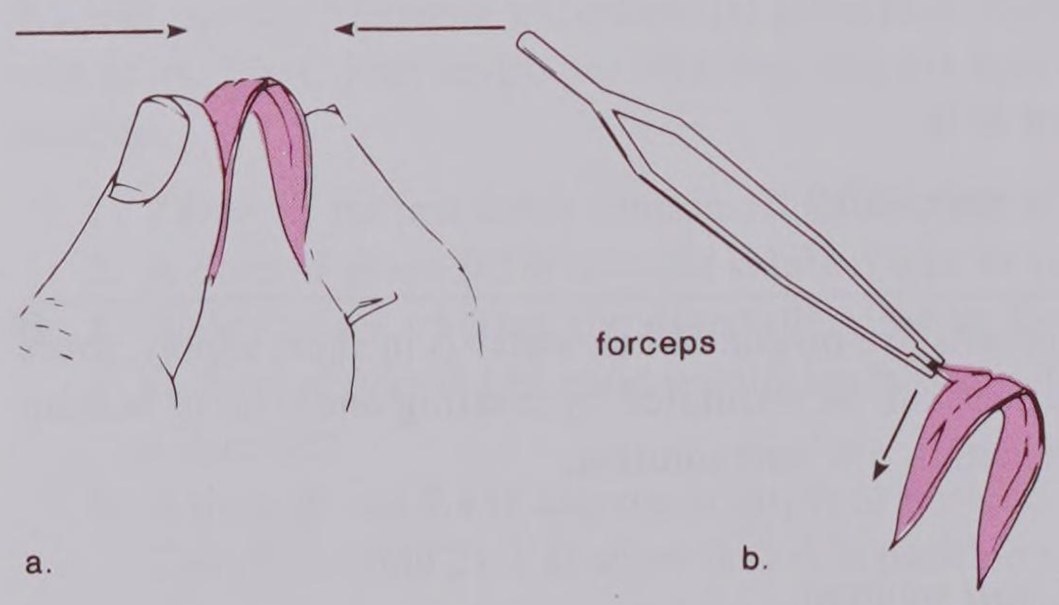


Figure 21.3 Technique for removing epidermis from underside of a leaf. ***a.*** Pinch leaf. ***b.*** Remove epidermis with forceps.



Stomata

When the stomata are open, transpiration is occurring at its most rapid rate (fig. 21.2).

Number of Stomata

- Ⓢ 1. Obtain a *leaf* from a plant designated by your instructor.
- Ⓢ 2. Have a slide with a drop of distilled *water* ready.
- Ⓢ 3. Using the technique demonstrated in figure 21.3., obtain a strip of *epidermis* from the underside of the leaf, and mount it in the drop of water, outer side up.
- Ⓢ 4. Add a coverslip and examine using a compound light microscope under low power (10×), then *high* power (45×).
- Ⓢ 5. Find a portion of the mount that is free of air bubbles and estimate the *number* of stomata visible in the field at one time. _____
- Ⓢ 6. Use the diameter (d) of field, in millimeter, you determined for high power in Laboratory 1, calculate the area you are looking at (area of a circle = πr^2). _____ mm^2 (Reminder: r=d/2)
- Ⓢ 7. Divide the number of stomata per field by the area of the field in mm^2. _____ stomata/mm^2
- Ⓢ 8. Compare your results to the data in table 21.2 for the number of stomata per mm^2 on leaves of other plants. To which species does yours come closest? _____

Table 21.2 Stomata per Square Millimeter of Leaf Area

Species	Lower Surface
Abies balsamea (balsam fir)	228
Lilium bulbifera (lily)	62
Morus alba (white mulberry)	480
Syringa vulgaris (lilac)	330
Nymphea alba (waterlily)	0
Pinus strobus (white pine)	0
Helianthus annuus (sunflower)	325
Lycopersicon esculentum (tomato)	130
Phaseolus vulgaris (bean)	281
Triticum sativum (wheat)	14

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9. Recall, or look at a metric ruler to determine, how small one millimeter actually is.

The same researcher referred to earlier found that the average leaf area was 26 cm^2 (2,600 mm^2). According to your data, would you expect each leaf to have a large number of stomata and, thus, expect a plant to tend to lose a great deal of water by means of transpiration? _____

Guard-Cell Response

Stomata are easily recognizable because of the surrounding guard cells.

How do guard cells differ from the surrounding epidermal cells in your slide? _

_____

Because of the anatomy of guard cells, the stoma is *open* when the cells are *turgid*. When water is in short supply, these cells become *flaccid* and collapse, *closing* the stoma. These conditions can be simulated by treating one strip of bottom epidermis containing guard cells with *distilled water* and another with a *5% salt* solution.

S 1. Have a slide with a drop of *5% salt* solution ready.
S 2. Obtain another strip of epidermis and mount it in the *5% salt* solution.
S 3. Wait 5 minutes, then observe this slide and compare the appearance of the guard cells in this slide to that in the slide you made previously. *Describe* the difference. _____

_____

Do the stomata open when there is plenty of water available to the plant or when water is in short supply? ____

_____

Root Hairs

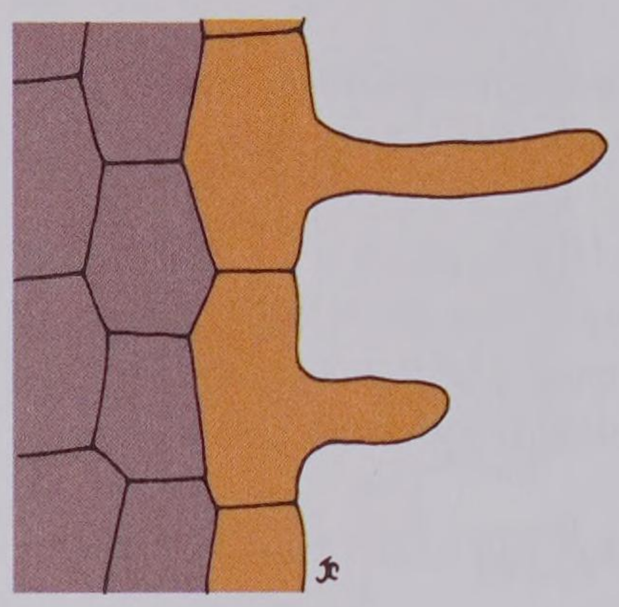
S 1. Examine a young *germinated* seed. Note that the root is elongating most rapidly and has grown some distance beyond the seed coat.
S 2. Using a binocular dissecting microscope, locate the *root cap,* the *root tip,* and the region where the *root hairs* have formed on the root surface.
S 3. Be sure that the seedling is kept *moist* on wet filter paper in a petri dish while you are looking at it.

Structure

1. Remove the root from the seed and make a wet mount of the root, using water and/or a solution of *0.1% neutral red*.
2. Observe your slide under the microscope.
3. Complete the drawing by filling in the nucleus. What cells give rise to root hairs? _____

   Do root hairs have cross walls? _____

   How many cells can you see in a single root hair? _____



Absorption of Water

We will use the following experimental procedure to determine under what osmotic conditions water is likely to enter root hairs. First, your instructor will demonstrate that a less-dense sucrose solution will float on a more-dense sucrose solution.

1. *Three* 15 ml test tubes contain 10 ml of clear *0.2M sucrose* solution each.
2. A drop of *green 0.2M sucrose* solution will be released about midway in one of these tubes.
3. Watch to see whether the drop falls, rises, or becomes dispersed.
4. A drop of *blue 0.1M sucrose* solution is similarly released in the second tube. Does it fall, rise, or become dispersed?
5. A drop of *red 0.4M sucrose* solution is released in the third tube. Does it fall, rise, or become dispersed? Complete table 21.3 to show that it is possible to relate density to tonicity (see definition in the next section).

Table 21.3 Relationship between Density and Tonicity

Test Tube	Movement of Drop	Compared to 0.2M Sucrose	
		Density of Drop	Tonicity of Drop
0.2M	Dispersed	Same	Isotonic
0.1M			
0.4M			

Osmosis in Potatoes

1. Set up 2 sets of 6 test tubes each in a test tube rack. Label the first set 1a, 2a, 3a, 4a, 5a, and 6a. Label the second set 1b, 2b, 3b, 4b, 5b, and 6b.
2. Fill tubes 1a and 1b with a solution of *0.15M sucrose.*
   Fill tubes 2a and 2b with a solution of *0.2M sucrose.*
   Fill tubes 3a and 3b with a solution of *0.25M sucrose.*
   Fill tubes 4a and 4b with a solution of *0.3M sucrose.*
   Fill tubes 5a and 5b with a solution of *0.35M sucrose.*
   Fill tubes 6a and 6b with a solution of *0.4M sucrose.*
3. Add a small crystal of *methylene blue* dye to the *a* series of tubes.
4. Using a cork borer, remove *cylinders* of tissue from a large *potato.* Cut the cylinders into uniform 2 cm lengths with a razor blade cutter until you have 18 sections. As you cut them, place them under a damp paper towel until you have them all. Cut all sections from the *same* potato.
5. Drop 3 sections of potato into each test tube containing a *clear sucrose* solution (the *b* series).
6. After at least 20 minutes, remove the potato cylinders from the tubes by stabbing with a dissecting needle.
7. Beginning with tube 1a, remove a small amount of the *dyed solution* with a dropper. Using the technique demonstrated by your instructor, release a drop of this solution in the corresponding b series tube. Record in table 21.4 whether the drop rises, falls, or disperses near the tip of the pipette.
8. On the basis of these results, determine whether water went *into* the potato slice or *out of* the potato slice. Record your determinations under "movement of water" in table 21.4.
9. On the basis of your determinations of the movement of water, state whether the original solution was *isotonic, hypotonic,* or *hypertonic.*

Recall from Laboratory 2 or your text reading that *hypotonic* solutions have a *lower* concentration of solute (salt), and higher concentration of solvent (water), therefore, water enters *into* cells immersed in them. *Hypertonic* solutions have a *higher* concentration of solute (salt) and lower concentration of solvent (water), therefore, water *exits* from cells immersed in them.

Table 21.4 Experimental Results

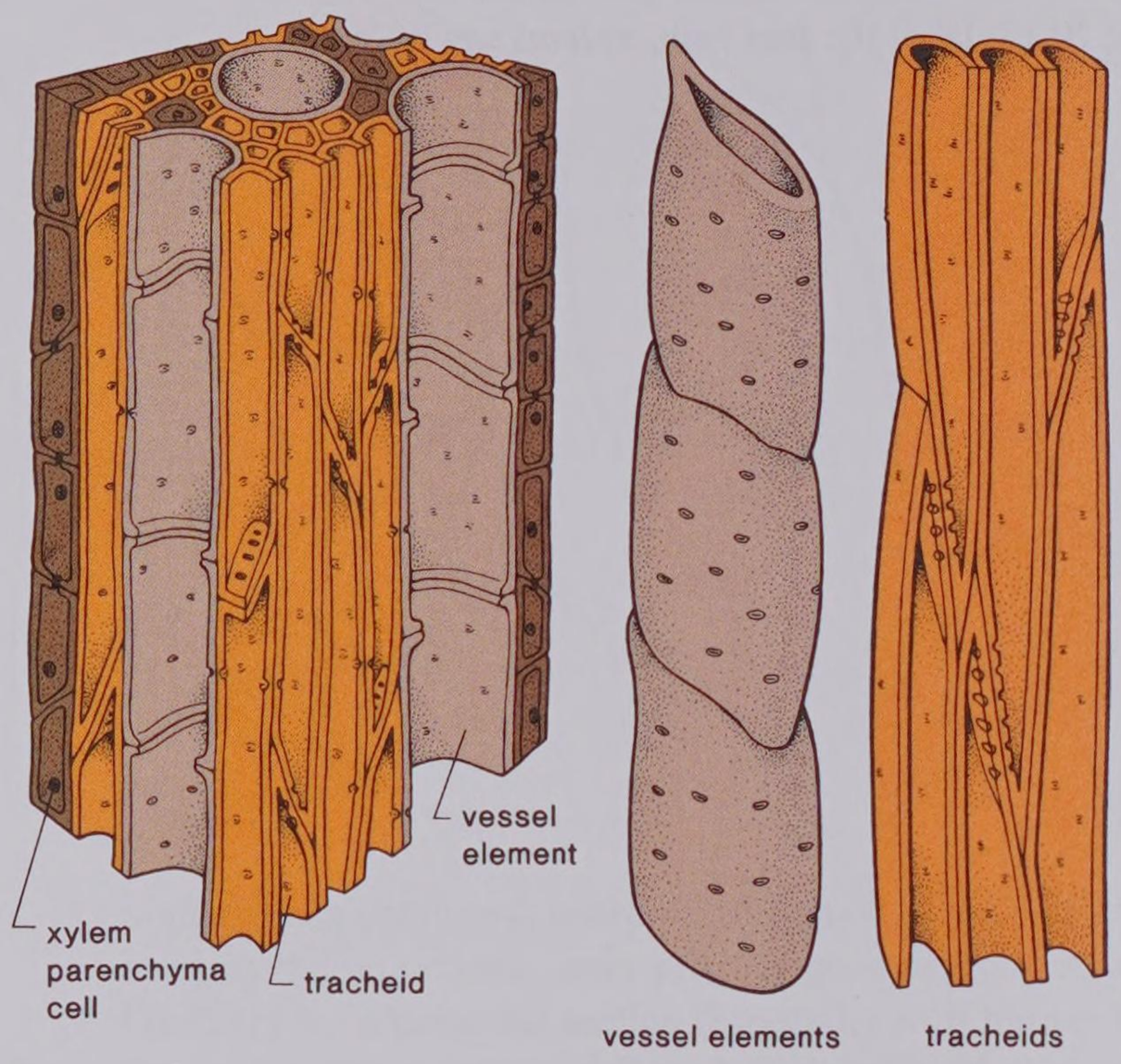
Test Tube	Movement of Drop	Movement of Water (into or out of potato)	Tonicity of Original Solution
1*b*			
2*b*			
3*b*			
4*b*			
5*b*			
6*b*			

This experiment shows that only if groundwater is _____to cytoplasm in root hairs will root hairs be able to absorb water. If by chance a plant's roots are surrounded by a hypertonic solution, the roots will _____water.

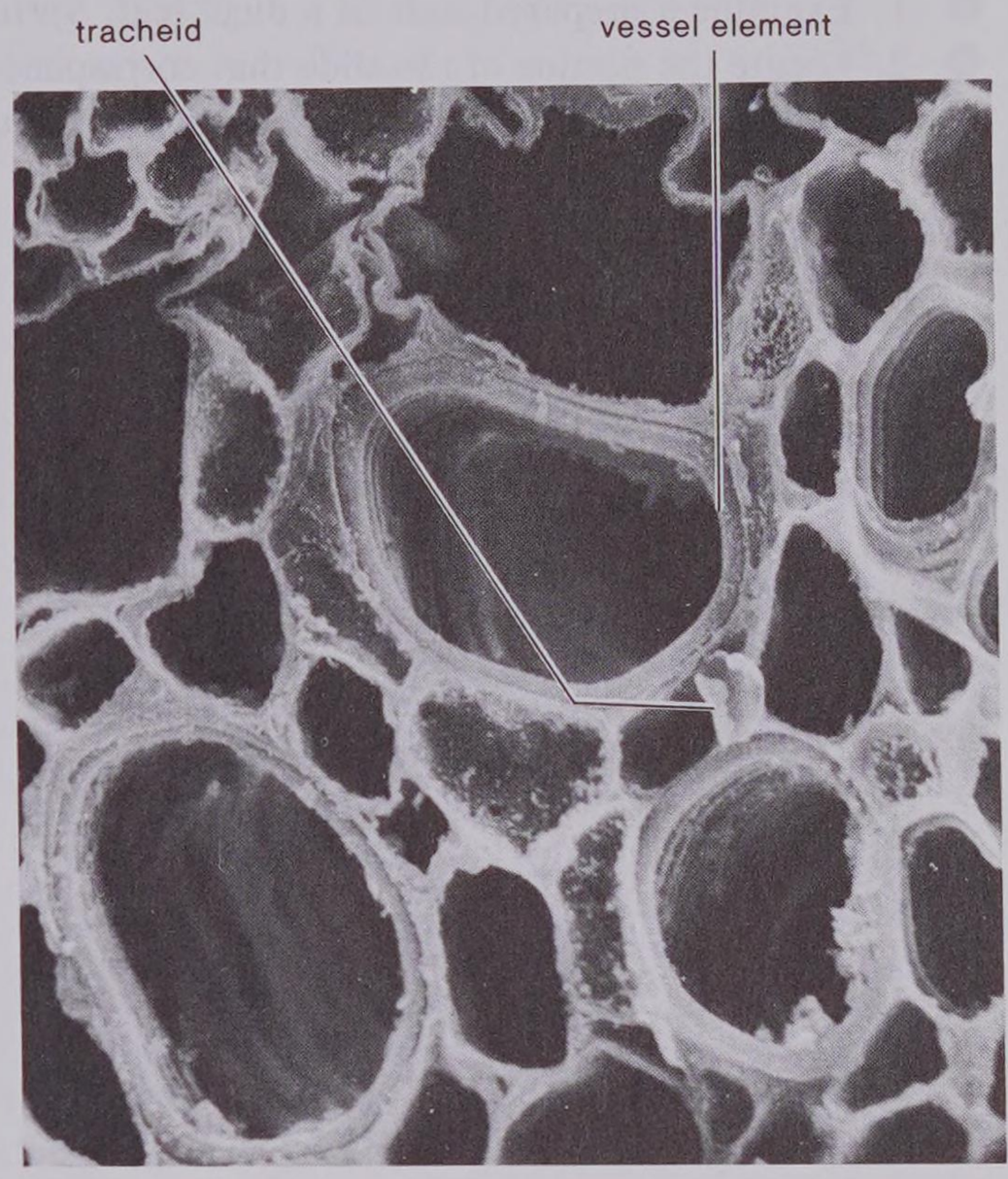
Water Transport Tissue

After water has entered the tissues of a root, it traverses the root to the vascular cylinder, where it enters the xylem. Figure 21.4 shows that xylem contains 2 types of conducting cells: *vessel elements* and *tracheids.* Since vessel elements lack end walls, they form a continuous pipeline from the roots to the leaves.

Figure 21.4 Xylem structure. ***a.*** Diagram of longitudinal section. ***b.*** Scanning electron micrograph of cucumber root xylem.



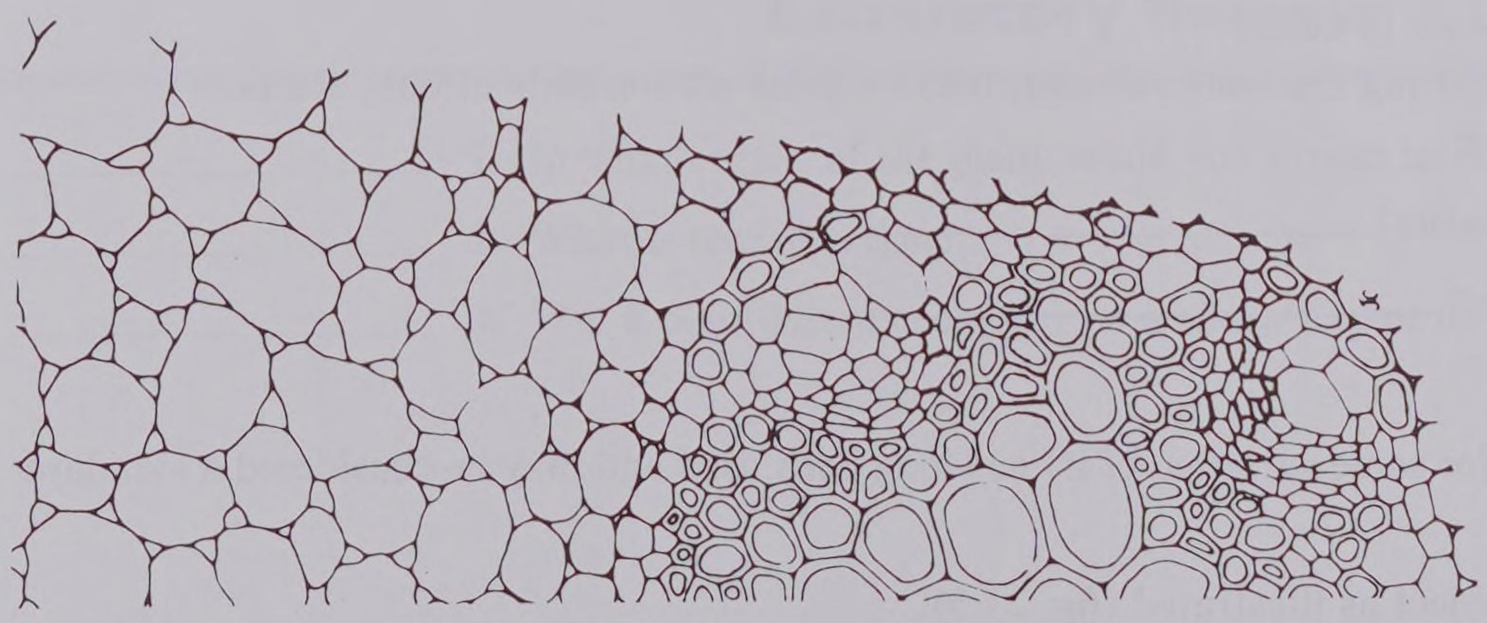
a.



b.

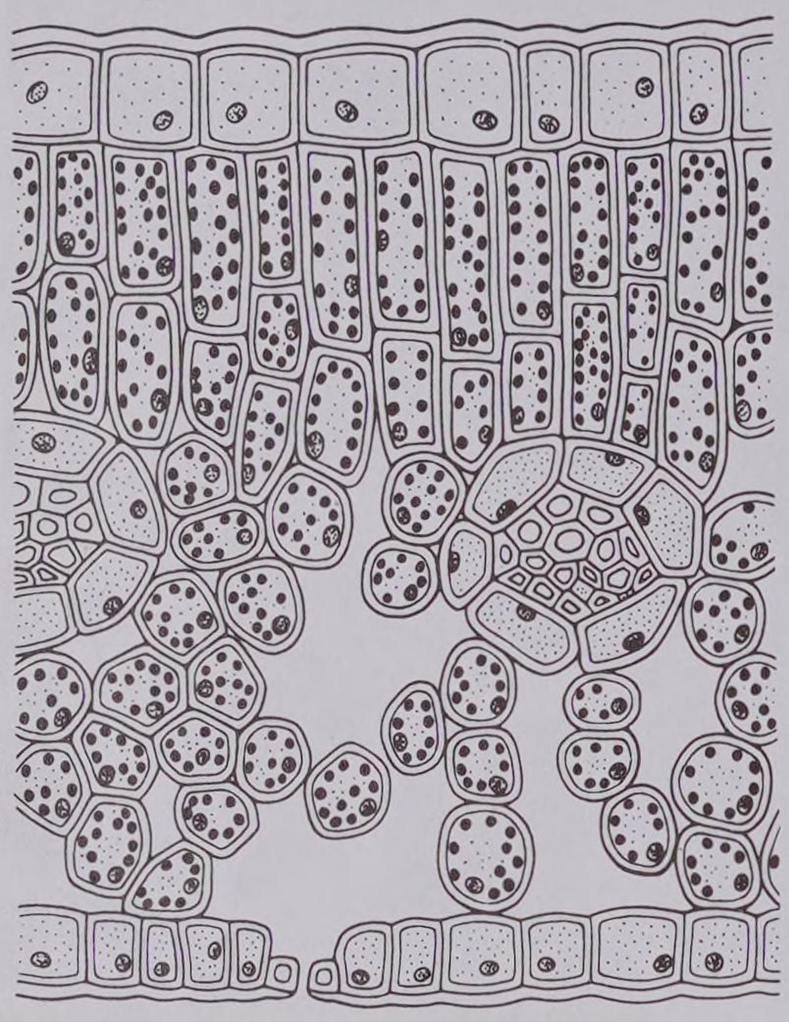
Root Cross Section

1. Examine a prepared slide containing young and old cross sections of buttercup (*Ranunculus*) root.
2. Locate the cross section that corresponds best to the illustration.
3. With the help of figure 20.5, label the *vascular cylinder, xylem,* and *phloem.*



Leaf Cross Section

1. Examine a prepared slide of a dicot leaf. *Syringa* (Lilac), *Liqustrum* (Privet), or _____
2. Locate the portion of the slide that corresponds best to the cross sectional view of the illustration.
3. Identify the leaf vein and, with the help of figure 20.13, label the *leaf vein, xylem,* and *phloem.*



Rise of Transpiration Stream

Water continually evaporates at the leaves as transpiration occurs. This creates a force that pulls on the column of water within the xylem, and thus water rises in the xylem.

On display are 2 stalks of celery, one stalk with its cut end placed in a beaker containing a small amount of *methylene blue solution,* and the other stalk with its cut end placed in *water.*

1. Observe each stalk and verify that the *methylene blue* solution has risen to the leaves (note that the tips of the leaves are blue).
2. Obtain a piece of stem from each stalk of celery.
3. Following the directions given for cutting freehand sections, make a cross section slide of both stalks.
4. Observe them under the microscope.

   Is the dye located in the vascular tissue? _____

   Is it located in the xylem or phloem? _____

Cutting Freehand Sections*

This method of cutting freehand sections (or some variation of it) has long been used and may be considered a standard procedure

1. Firmly grasp stem, root, or other object as illustrated (fig. 21.5).
   a. Brace both elbows solidly against ribs to steady hands.
   b. Use a new and *sharp* single-edged, backed razor blade (Gem or similar).
   c. Rest blade flat on left index finger as shown.
   d. By wrist and forearm action, pull your right hand with the blade toward you across the stem or other object to cut the sections by a smooth, continuous slicing motion, using as much edge length as possible. Pull the blade to slice through tissues; don't push and don't chop.
   e. Keep left thumb and knuckle well below the cutting edge.

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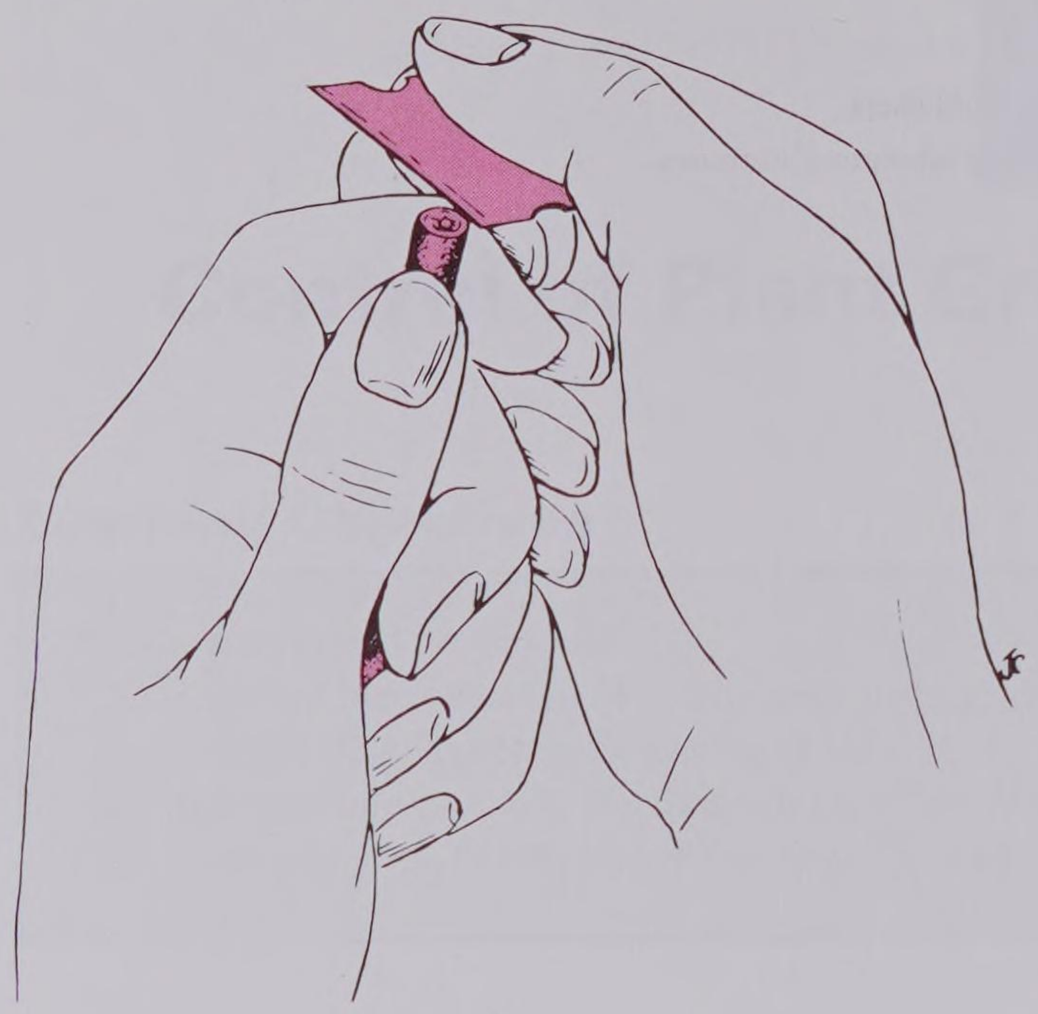
Figure 21.5 Method of cutting a freehand section.



2. Sometimes a combined, coordinated motion of both hands—the right pulling toward you and the left moving away at the same time—may produce good sections. Always *slice,* don't chop!
3. Don't try for a complete section (especially with harder materials).
   Attempt to cut a narrow, wedge-shaped section that tapers off to a very thin edge ("feather edge"). Thinner edges allow for more ease in viewing cellular details under the microscope.
4. a. Always cut many more sections than will be needed at one time.
   b. Float off sections into a watch glass of *water* or place directly on a microscope slide.
   c. Push off adhering sections with a dissecting needle or small brush.
   d. Pick out the best (thinnest) sections.
   e. Place on a slide in a drop of *water,* and cover with a cover glass for observation under the microscope.

Laboratory Review 21

_____ 1. In what organs of the plant would you expect to find vascular tissues?

_____ 2. What is transpiration?

_____ 3. Will a plant lose more water on a sunny day or on a cloudy day? Why?

_____ 4. What happens to most of the water that enters the plant?

_____ 5. What cells within the xylem conduct water most efficiently?

_____ 6. When water is in short supply, what happens to the stomata? How does this help the plant survive?

_____ 7. A piece of tissue is placed in a 0.2M sucrose solution. After the tissue is removed, a 0.2M drop rises. What is the tonicity of the solution compared to the drop?

References

Dean, H. L. 1982. *Biology of Plants*. Dubuque, IA: Wm. C. Brown Publishers.

Dolphin, W. D. 1983. *Biology Laboratory Manual*. Dubuque, IA: Wm. C. Brown Publishers.

DiLiddo, Rebecca D. Estimation of solute concentration in plant tissue. Unpublished laboratory exercises.

Laboratory

22

Control of Plant Growth and Development

Learning Objectives

Students should be able to

1. describe the response of stems and roots to gravity;
2. describe the response of stems to light;
3. describe one possible mechanism of action of plant hormones;
4. relate the growth responses observed to appropriate hormonal actions and/or phytochrome.

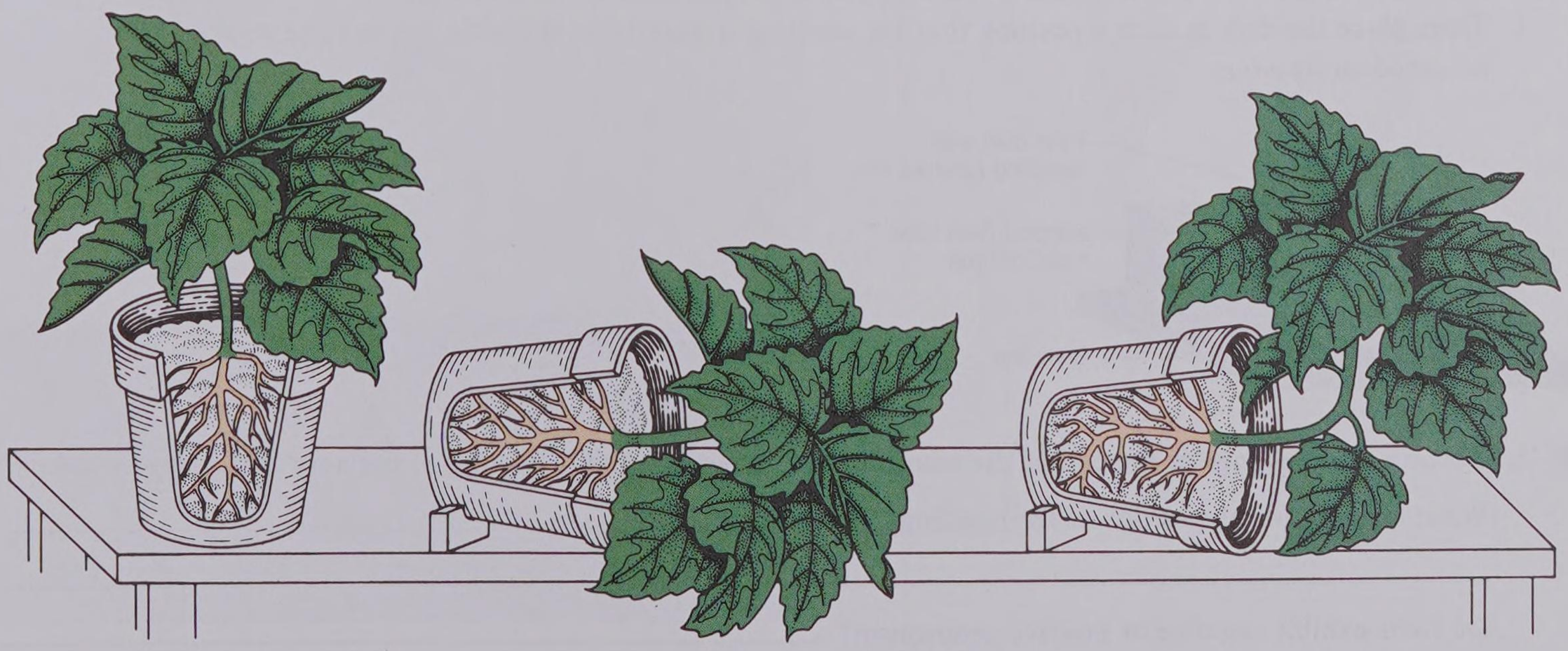
Introduction

Plant growth and development are controlled by environmental stimuli such as light and gravity. Such stimuli promote changes in cell permeability, hormone levels, enzyme activity, and a variety of other alterations that enable a plant to respond to the stimulus. The response of a plant is often observed as a change in its growth pattern. We will observe some of these changes in the laboratory today.

Tropisms

Plant growth occurs in a particular direction in response to a stimulus. This directional growth is called a *tropism*. Growth in the direction of the stimulus is said to be a positive tropism. Growth away from a stimulus is a negative tropism (fig. 22.1). Gravity and light are 2 stimuli that can cause tropic responses in plants.

Figure 22.1 When an upright plant is turned on its side, the roots bend down and the stem bends up with time. Thus, roots demonstrate positive geotropism and stems demonstrate negative geotropism.



Geotropism

Geotropism is the bending response of a plant organ to the stimulus of gravity.

Geotropism of Stems

Ⓢ 1. Place a potted sunflower plant 15–20 cm tall in a *horizontal* position on the laboratory table.
Ⓢ 2. Carefully measure the shortest distance from the tip of the stem to the surface of the table.
Ⓘ Ⓢ 3. Repeat this measurement every 15 minutes.
Ⓢ 4. Record your data in table 22.1.

Table 22.1 Geotropism in Stems

Tim	Distance from Desktop	Comments
15 min		
30 min		
45 min		
60 min		
75 min		
90 min		

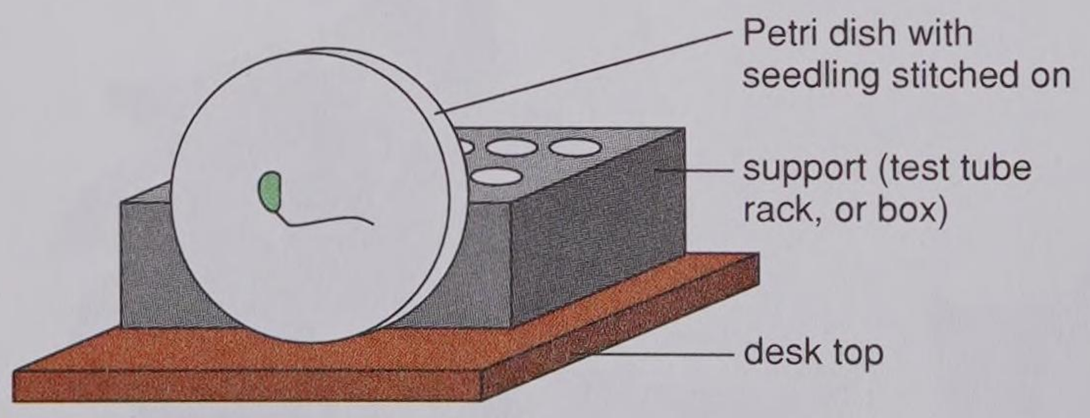
Ⓘ Ⓢ 5. After 90 minutes, note any bending of the stem and locate the region of curvature.
Ⓢ 6. Compare this with the location of the region of curvature in a plant that has been lying in a horizontal position for 24 hours. What are the differences between the 2 plants? _____

Suggest a possible explanation for these differences. _____

_____

Geotropism of Roots and Stems

Ⓢ 1. Carefully *fasten* straight, uninjured germinated seeds of mustard, radish, or cress to a disc of filter paper (several sheets) with a needle and thread. Place the assembly in a covered petri dish.
Ⓢ 2. Make sure that the paper is *wet* (not dripping), and then seal the dish with tape.
Ⓢ 3. Mark on the glass (or plastic) with a marking pencil the *position* of the seedlings.
4. Then, place the dish in such a position that the seedling is parallel to the table top and the petri dish is balanced on its *edge:*



Ⓣ 5. Observe the seedlings at the end of the laboratory period and at the beginning of the next laboratory period.

What changes in the position of the seedlings are evident? _____

_____

Do roots exhibit negative or positive geotropism? _____

Do stems exhibit negative or positive geotropism? _____

Phototropism (Demonstration)

Phototropism is the bending response of a plant to the stimulus of light.

Pots of Seedlings

Pots of oat, radish, or wheat seedlings were grown until they were 4–5 cm tall. One pot was placed in a box that had a 7 mm opening on one side. Another pot was placed in a box that had no opening. The plants were kept in darkness overnight until the beginning of the laboratory period. Then, both boxes were placed in sunlight. Sunlight should have directly entered the opening in the first box.

Ⓢ Just before the end of the laboratory period, the boxes should be removed from the seedlings. Do stems exhibit negative or positive phototropism? _____

_____

Redtop Seedlings

Seedlings of redtop were grown 1.2–2 cm tall on the surface of tap water that almost filled a glass bowl.

Ⓢ 1. Expose one bowl of seedlings to strong one-sided illumination by placing the bowl on a windowsill.

Ⓢ 2. Place another bowl 8–10 m away from the window in subdued light. What happens after 2 hours? _____

_____

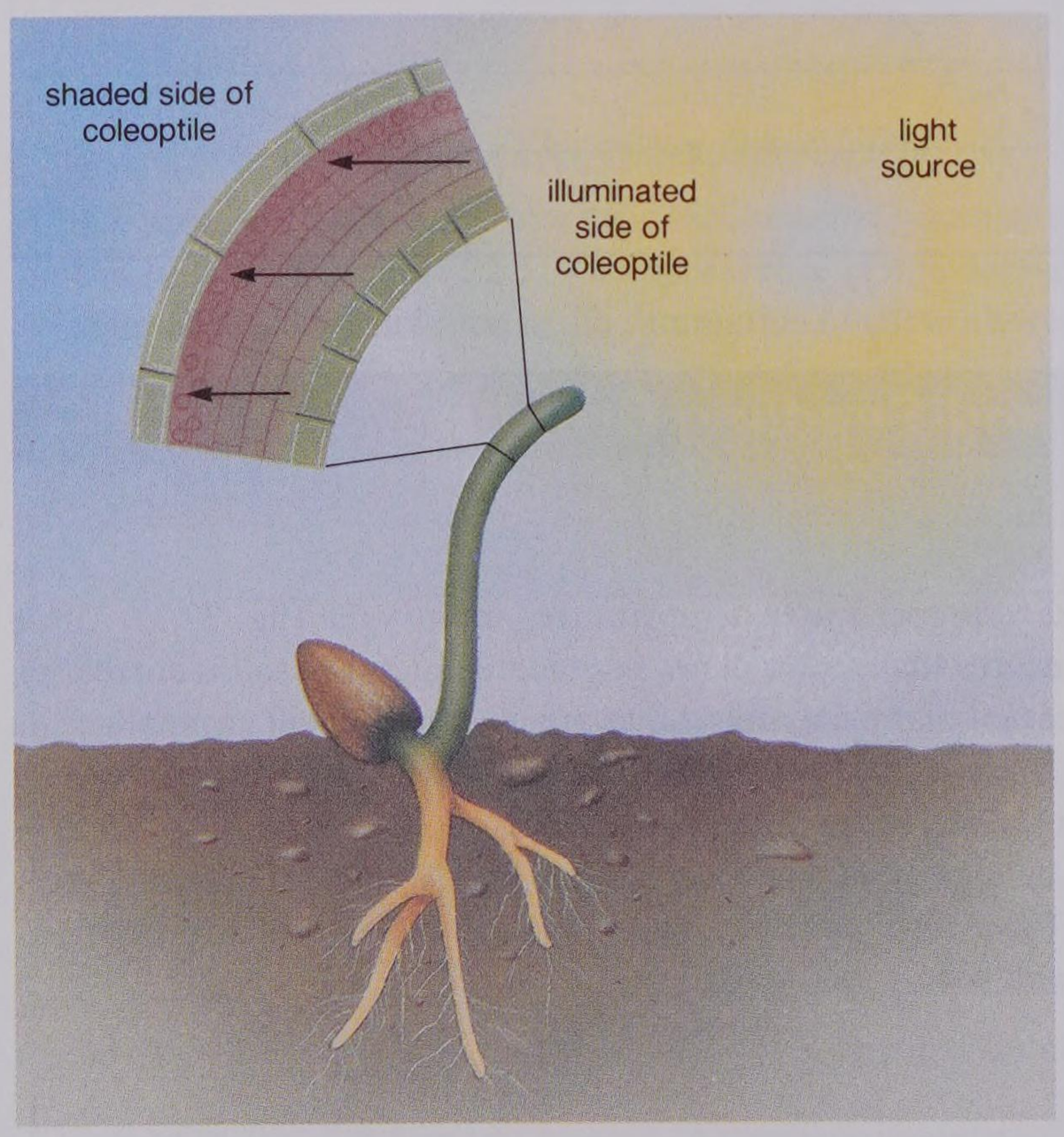
Conclusion

In a stem lying horizontally that is bending in response to gravity, which will elongate faster: the side closest to the earth or the side away from the earth? _____

In a stem responding to a unidirectional light source, which will elongate faster: the side closest to the light or the side farthest from the light? _____

As figure 22.2 shows, it is elongation of cells that causes the bending we have observed in a stem. The hormone *auxin* has long been implicated in this process.

Figure 22.2 Elongation of cells on the shady side of a stem causes bending toward the light.



Role of Hormones (Demonstration)

Plant hormones can affect cells both directly and through enzyme systems. The line between these effects is not always clear, and most processes probably involve a combination of *hormonal* and *enzyme* controls. *Gibberellic acid* (GA) is a hormone that has been found to turn on the activity of the enzyme *alpha-amylase.*

(D) A *demonstration* on display has 5 petri dishes containing *starch agar,* some containing *oat* or *wheat seeds* that have been cut in half.

Petri dishes:

1. Each seed half has an *embryo.* Alpha-amylase breaks down the starch, resulting in a clear zone around the seeds. Is there evidence of alpha-amylase activity?

(S) Record your answer in table 22.2.

2. Each seed half *lacks* an *embryo.* Is there evidence of alpha-amylase activity?

(S) Record your answer in table 22.2.

3. Each seed half *lacks* an *embryo* but each half was treated with a drop of *gibberellic acid.* Is there evidence of alpha-amylase activity?

(S) Record your answer in table 22.2.

4. Only drops of *gibberellic acid* were placed on the agar plate. Is there evidence of enzyme activity?

(S) Record your results in table 22.2.

5. This is the control plate with *no seed* halves or *treatment.*

(S) Record your results in table 22.2.

Table 22.2 Role of Hormones

Petri Dish (content)	Clear Zone (present or absent)	Enzyme Activity (−, +, ++, +++)	Explanation of Enzyme Activity
1			
2			
3			
4			
5			

Seed Germination and Seedling Growth

Seed Germination (Demonstration)

Some seeds require a light treatment in order to respond to proper water, temperature, and oxygen (fig. 22.3). These seeds will remain dormant unless exposed to light. This ensures that they will not germinate unless the soil is stirred up, uncovering the seeds. When seeds are dropped from the parent plant, only those that are not covered will germinate; the others are left to germinate at a future time or to die. The seeds of such plants are often very small and so must germinate on or near the surface or they will run out of food before the plants reach the light, where photosynthesis may begin.

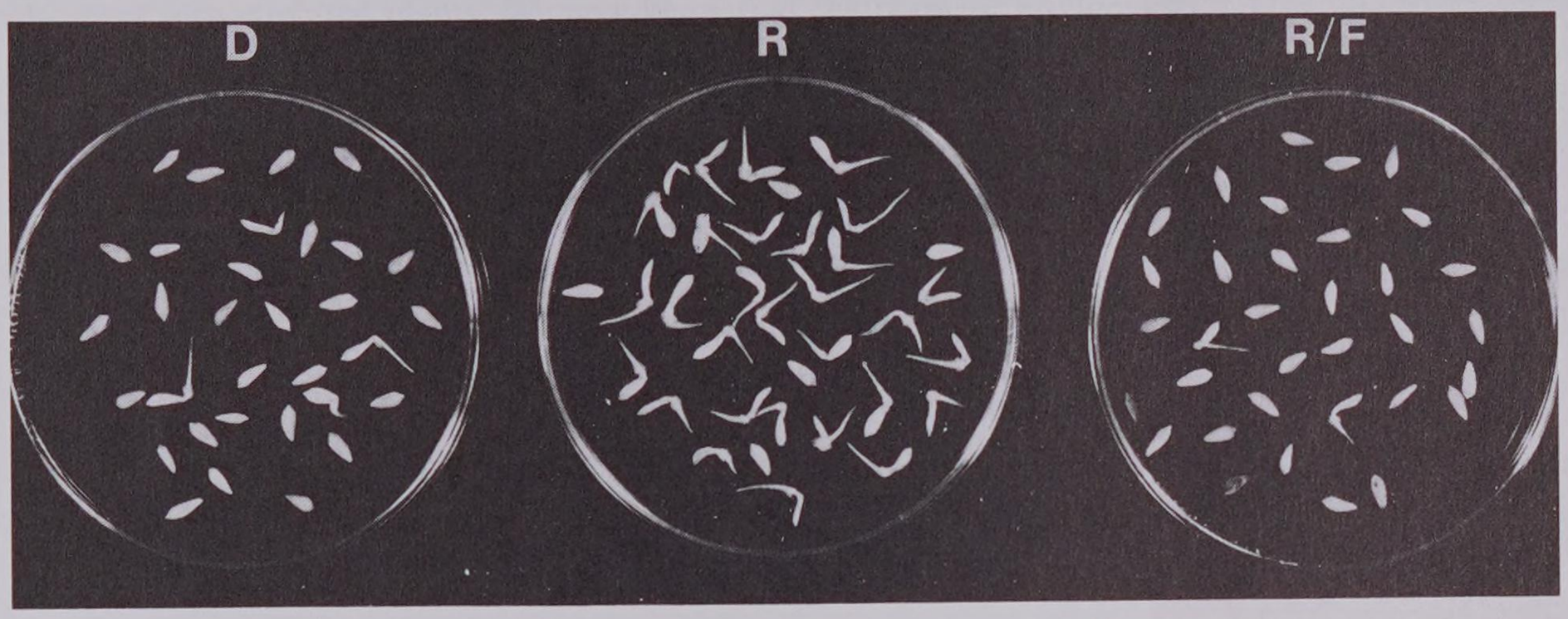
(D) Each of 2 petri dishes contains 25 lettuce seeds on a piece of wet filter paper. One dish, marked "dark," was immediately wrapped in foil and placed in the dark. The other dish, marked "light," was exposed to ordinary daylight. (Filter paper will dry out, so do not place in bright light and keep paper moist.)

(S) 1. Count the number of seeds that have germinated in each dish.

Dish marked *dark:* _____

Dish marked *light:* _____

Figure 22.3 Lettuce seed germination experiment. ***a.*** Few lettuce seeds germinate when the petri dish is kept in the dark (D). ***b.*** Germination occurs when seeds kept in the dark are exposed to red light (R). ***c.*** Germination is again inhibited if the flash of red light is followed by a flash of far-red light (R/F). (From *Phytochrome and Plant Growth* by K. E. Kendricks and B. Frankland. Published by Edward Arnold, LTD.)



a. b. c.

Ⓢ 2. Count the number of lettuce seeds that have germinated in the petri dishes from the experiment shown in figure 22.3.

D (dark) _____

R (red light) _____

R/F (far-red) _____

3. Describe how your data compares to this data. _____

_____

Is your data similar or dissimilar? _____

Explain: _____

_____

Seedling Growth (Demonstration)

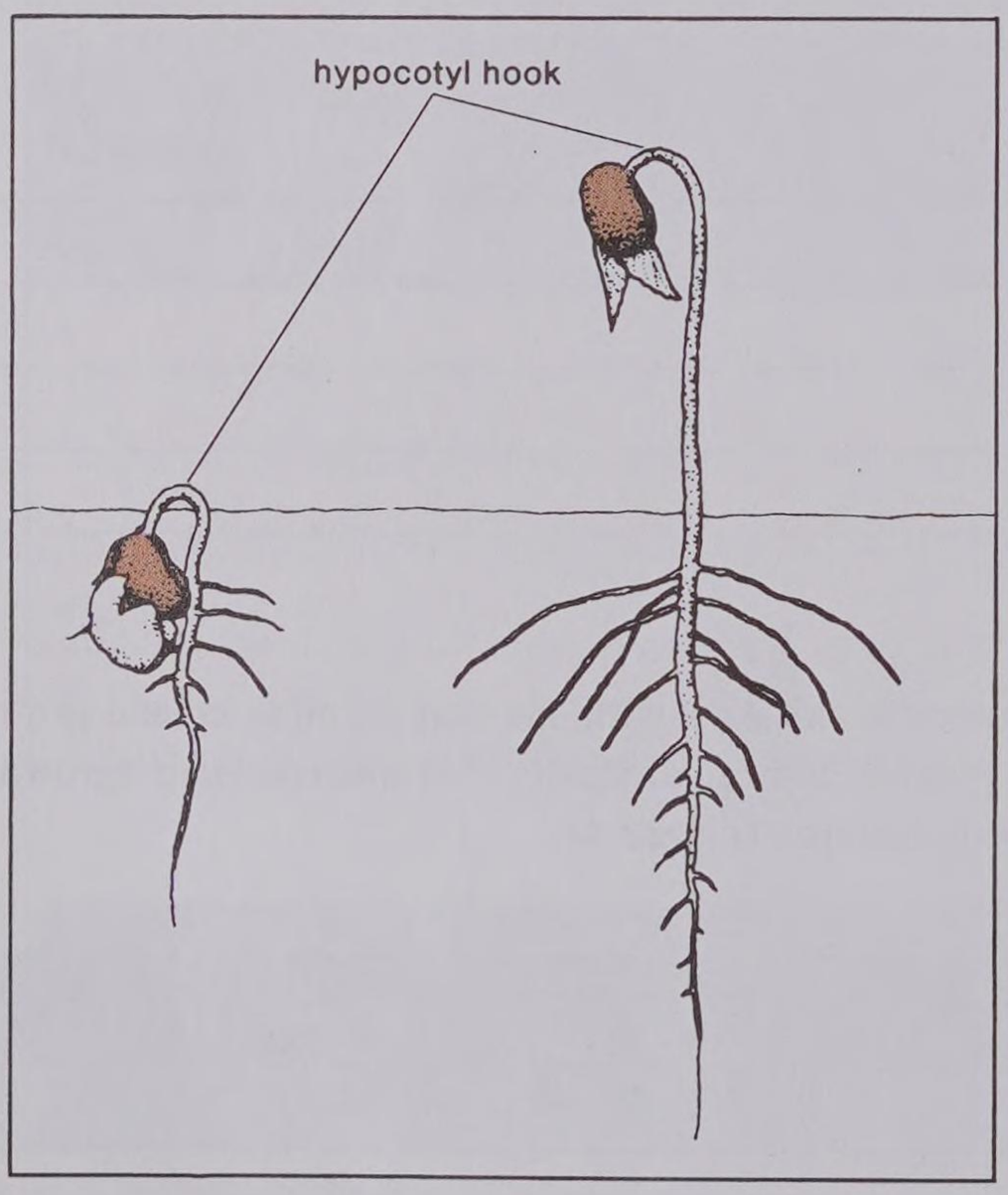
Ⓢ Compare the appearance of a bean seedling that germinated and grew in the *dark* with one that germinated and grew in the *light*. Fill in table 22.3. The appearance of the dark-grown plant is typical of plants that are *etiolated* (grown without sunlight). Etiolation is the summation of these altered growth patterns (fig. 22.4).

Table 22.3 Comparison of Normal and Etiolated Plants

	Normal	Etiolated
Degree of hypocotyl hook		
Color of stem		
Color of leaf		
Length of stem		
Size of leaf		
Stiffness of stem		
Other comments, if any		

Figure 22.4 Bean seedlings on the left are etiolated because they were grown in the dark. Bean seedlings on the right are much shorter with well-formed leaves because they were grown in the light.

Figure 22.5 Seedling of a lima bean showing hypocotyl hook. (Copyright 1975 by Kendall/Hunt Publishing Company. Reprinted with permission.)



Ⓢ Notice that as a response to light, the leaves are lifted up above the ground, and chlorophyll is synthesized so that photosynthesis may begin. When the seedling is grown in the dark, the stem is elongated. Why is stem elongation under these circumstances possibly beneficial? _____

_____

Discussion

Figure 22.3 shows that exposure to 3 minutes of only *red* light can promote the germination of lettuce seeds kept in the dark. But if this exposure is followed by 3 minutes of *far-red* light, germination will not occur. Similar results are also observed with etiolated plants. We now know that the pigment *phytochrome* is involved in responses that are sensitive to red light/far-red light effects. Presumably, when red light is present (daylight contains red light), phytochrome initiates those physiological changes that bring about specific growth patterns.

Laboratory Review 22

_____ 1. What are the responses of the stem and root of gravity?

_____ 2. What is phototropism?

_____ 3. What hormone has been implicated in the responses of stems to light and gravity?

_____ 4. What happens to the cells on the shady side of a stem that causes the plant to bend toward the light source?

_____ 5. The hormone GA (gibberellic acid) activities what enzyme? When this occurs, what happens?

_____ 6. Do seedlings grown in the dark have green or white leaves?

_____ 7. Is etiolation adaptive?

_____ 8. Under what natural circumstances do you think etiolation would occur?

References

Dean, H. L. 1982. *Biology of Plants: Laboratory Exercises.* Dubuque, IA: Wm. C. Brown Publishers.

DiLiddo, Rebecca D. *Plant structure* and *function II—development.* Unpublished laboratory exercises.

Laboratory

23

Basic Mammalian Anatomy

Learning Objectives

Students should be able to

1. locate, identify, and give a function for the structures mentioned in regard to the fetal pig;
2. locate, identify, and give a function for the structures mentioned in regard to the human torso.

Introduction

The mammals you will dissect are fetal pigs. The period of pregnancy, or gestation, in pigs is approximately 17 weeks (compared to an average of 40 weeks in humans). The piglets used in class will usually be within 1–2 weeks of birth.

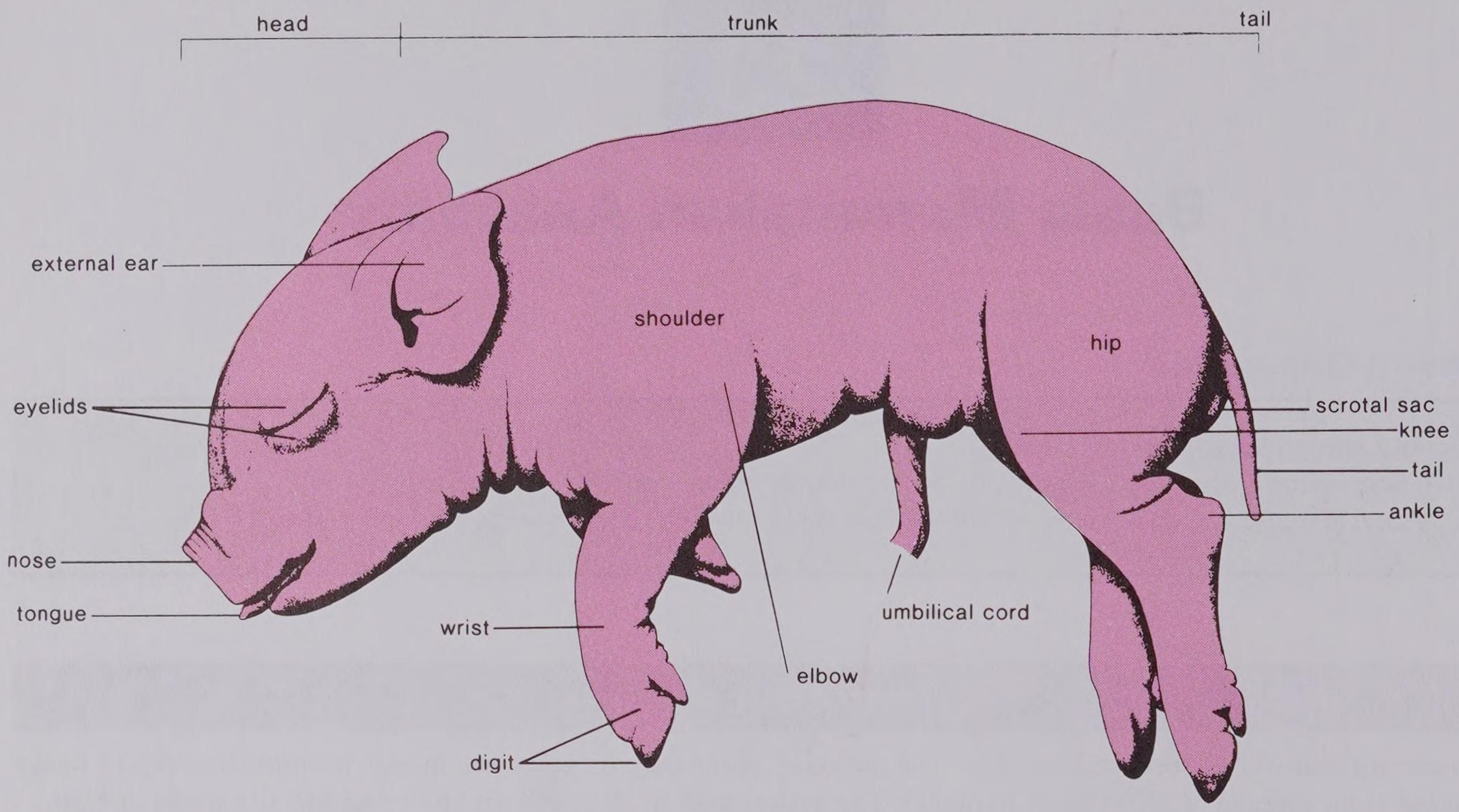
The pigs will have a slash in the right neck region indicating the site of blood drainage. A piece of cotton will most likely have been placed here. A *red latex* solution was injected into the *arterial system* and a *blue latex* solution was injected into the *venous system* of your pigs.

A number of anatomical terms may be used to describe the location of various parts of the body (table 23.1). You should become familiar with these terms and use them.

Table 23.1 Anatomical Terms

Dorsal—near or toward the back
Ventral—near or toward the belly
Lateral—near or toward the sides
Median—near or toward the middle
Anterior—near or toward the front end
Posterior—near or toward the hind end
Caudal—referring to the tail
Cranial—referring to the head
Longitudinal—in the axis from head to tail
Transverse—across the long axis
Superficial—on or near the surface
Pectoral—relating to the chest
Pelvic—relating to the hip region
Distal—far from
Proximal—close to

Figure 23.1 Fetal pig, external features.



Preparation

Ⓢ You will use a fetal pig for more than just one laboratory period, so your instructor will give you special instructions on *care, labeling,* and *storage.*

External Anatomy

Ⓢ 1. Place your animal in a dissecting tray and observe the several body regions: rather large *head,* short thick *neck,* cylindrical *trunk* with 2 pairs of *appendages,* and short *tail* (fig. 23.1).
Ⓢ 2. Examine the 4 legs and feel their various *joints* and *parts.*
Ⓢ 3. Determine which parts of the forelimb correspond to your *upper arm, elbow, lower arm, wrist,* and *hand.*
Ⓢ 4. Do the same for the *hind* limb, comparing it to your leg. Note that the pig is *hooved* and walks on its *toenails.*

Umbilical Cord

Ⓢ Note the *umbilical cord,* arising from the ventral (toward the belly) portion of the abdomen. Blood vessels, visible in their cut ends, carry nutrients, oxygen, and waste materials between the circulatory system of the embryo and the placenta, a special region of the uterus where maternal blood serves the needs of the fetus.

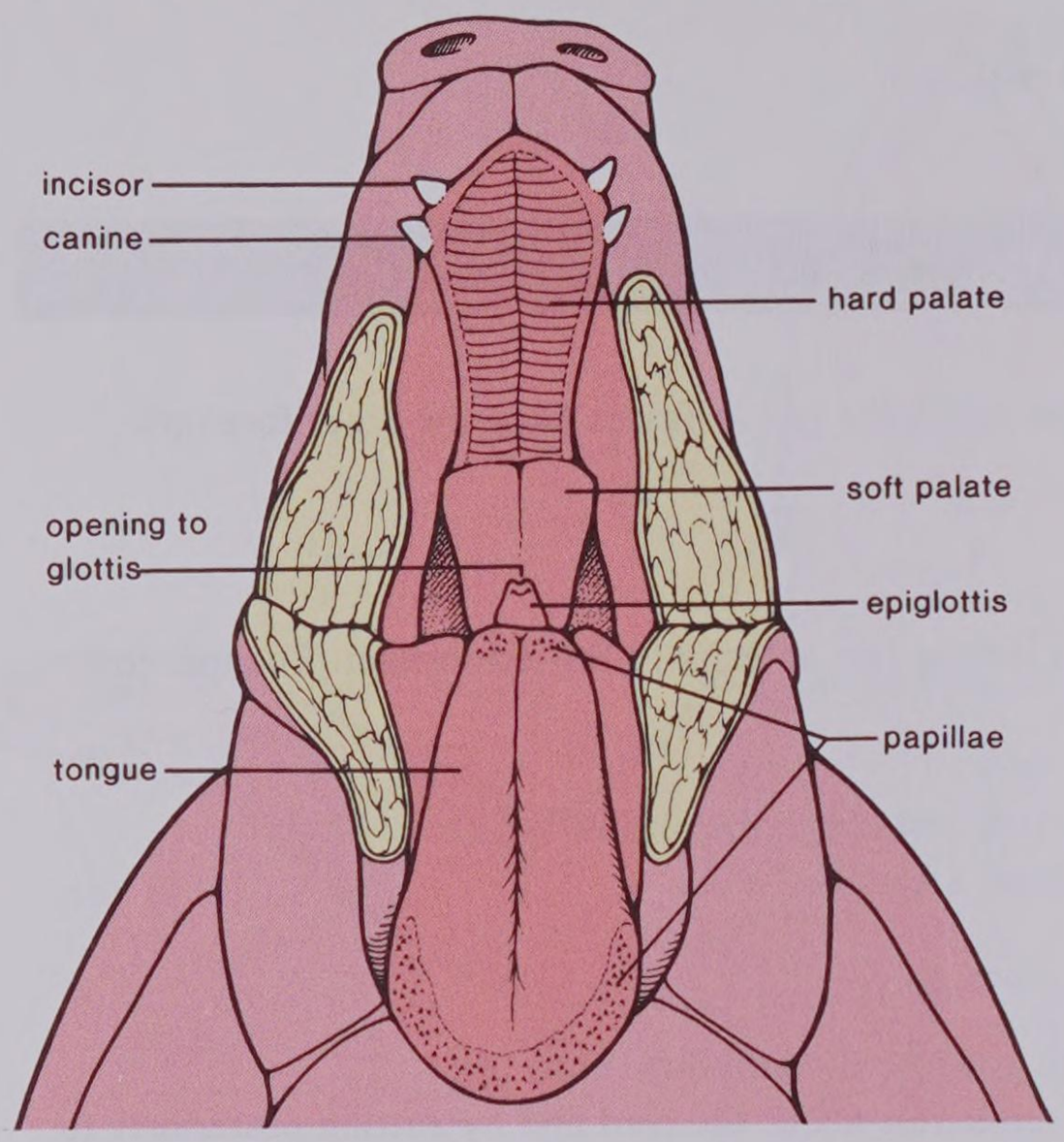
Nipples

Ⓢ Locate the small nipples. Note that nipples are *not* an indication of sex, since both males and females possess them. Nipples are the external openings from the *mammary glands,* a distinctive feature of mammals. *Hair* in some places on the body is another characteristic of mammals.

Sex

Ⓢ Just under the tail is the *anus,* the exterior opening of the digestive tract. In females, an opening that serves both genital and urinary systems, the *urogenital opening,* is located just anterior to the anus, and a small, fleshy papilla can be seen projecting from it. In females, the urinary and genital systems separate later in development and the single

Figure 23.2 Fetal pig, oral view.



opening becomes 2. In males, the single urogenital opening is located just posterior to the umbilical cord; the duct leading to it runs forward from between the legs in a long muscular tube, the *penis,* which can be felt under the skin.

What sex is your pig? _____

Oral Cavity

1. Insert a point of the scissors into one corner of the specimen's mouth and *cut* posteriorly (toward the hind end) for approximately 1½ inches.
2. Repeat on the opposite side. These cuts will enable you to push the lower jaw ventrally so that the *mouth* cavity can be examined.
3. Place your thumb on the tongue at the front of the mouth and gently push downward on the lower jaw. This procedure will tear some of the tissue in the angles of the jaws so that the mouth will remain partly open.

Tongue and Teeth

1. Look at the *tongue,* which is partly attached to the lower jaw region but extends posteriorly and is attached to a bony structure at the back of the mouth cavity.
2. Note small, undeveloped *teeth* in both upper and lower jaws. Other embryonic teeth may be found within the gums, since they have not erupted yet.

Hard and Soft Palate

Notice the ridge of the mouth; this is the *hard palate,* a tissue that separates the mouth cavity from the nasal passages dorsal to it. Posterior to the hard palate, the roof of the mouth becomes smooth; the smooth region is called the *soft palate.* In humans, the uvula is an extension of the soft palate that hangs down into the throat.

Pharynx

Push down on the tongue until you open the jaws far enough to see a slightly pointed flap of tissue pointing dorsally (toward the back) (fig. 23.2). This flap is the *epiglottis,* which covers the *glottis,* an opening through which air passes on its way to the lungs. Posterior and dorsal to the glottis, find the opening into the *esophagus,* the tube of the digestive tract that leads through the neck and thorax to join the stomach.

The *pharynx* is the most posterior part of the oral cavity and contains 3 openings, 2 of which, the glottis and the esophagus, you have located.

Ⓢ Now locate the *nasopharyngeal openings* to the nasal passages.

Incisions

Ⓢ 1. Place the fetal pig on its *back* in the dissecting pan.
Ⓢ 2. Tie a *cord* around one forelimb and bring it around underneath the pan to fasten back the other forelimb.
Ⓢ 3. Spread the *hind* limbs apart in the same way.

Thoracic Cuts

Ⓢ 1. With scissors always pointing up (never down), cut into the pig just anterior to the diaphragm. The incisions described following are numbered on figure 23.3.
Ⓢ 2. Cut straight up until you reach the hairs in the throat region. (*1*)
Ⓢ 3. Make 2 lateral cuts, one on each side of the midline incision just above the forelimbs (*2*).
Ⓢ 4. Pull back the flaps created by these cuts to expose the chest area (*3*).

Abdominal Cuts

Ⓢ 5. Cut into the pig just posterior to the diaphragm, straight down to the umbilical cord (*4*).
Ⓢ 6. Make a flap containing the umbilical cord by cutting a semicircle about the cord and by cutting posteriorly to the left and right of the cord (*6*).
Ⓢ 7. Make 2 cuts, one on each side of the midline incision just below the diaphragm (*6*).
Ⓢ 8. Make 2 more cuts, one on each side of the flap containing the umbilical cord just above the hind limbs (*7*).
Ⓢ 9. Pull back the side flaps created by these cuts to expose the abdominal area. To lift up the flap containing the umbilical cord, it will be necessary to cut the *umbilical vein.*

Neck Region

As a guide to your dissection, consult figure 23.4.

Thymus Gland

Ⓢ 1. Move the skin apart in the neck region just below the hairs mentioned earlier. If necessary, cut the body wall laterally to make flaps. You will most likely be viewing exposed muscles.
Ⓢ 2. *You have to cut through and clear away muscle* to expose the *thymus gland,* which lies among the muscles. This gland is believed to function in the fetus for the maturation of lymphocytes, the white blood cells responsible for immunity to disease.

Larynx, Trachea, and Esophagus

Ⓢ 1. Probe down into the deeper layers of the neck. Medially (toward the center), beneath several strips of muscle, is the hard-walled *larynx* and *trachea,* parts of the respiratory passage to be examined later in detail. Dorsal to the trachea find the esophagus.
Ⓢ 2. Insert a probe into the glottis and esophagus from the pharyngeal region, after opening the mouth.

Thyroid Gland

Ⓢ Locate the *thyroid gland* just posterior to the larynx, lying on top of the trachea. The thyroid is a gland that secretes a hormone, *thyroxin,* that regulates the basal metabolic rate.

Figure 23.3 Fetal pig, thoracic and abdominal incisions.

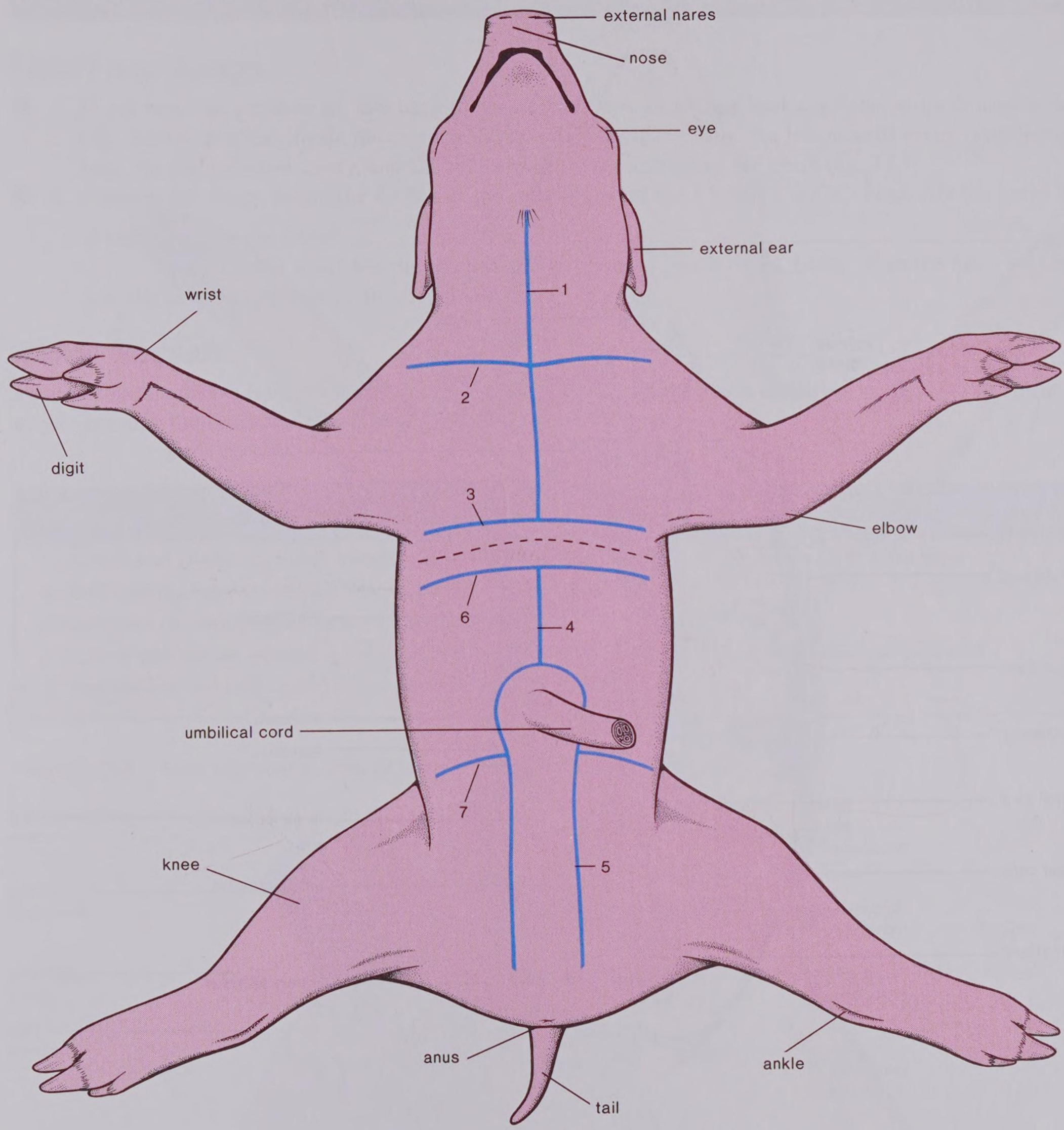
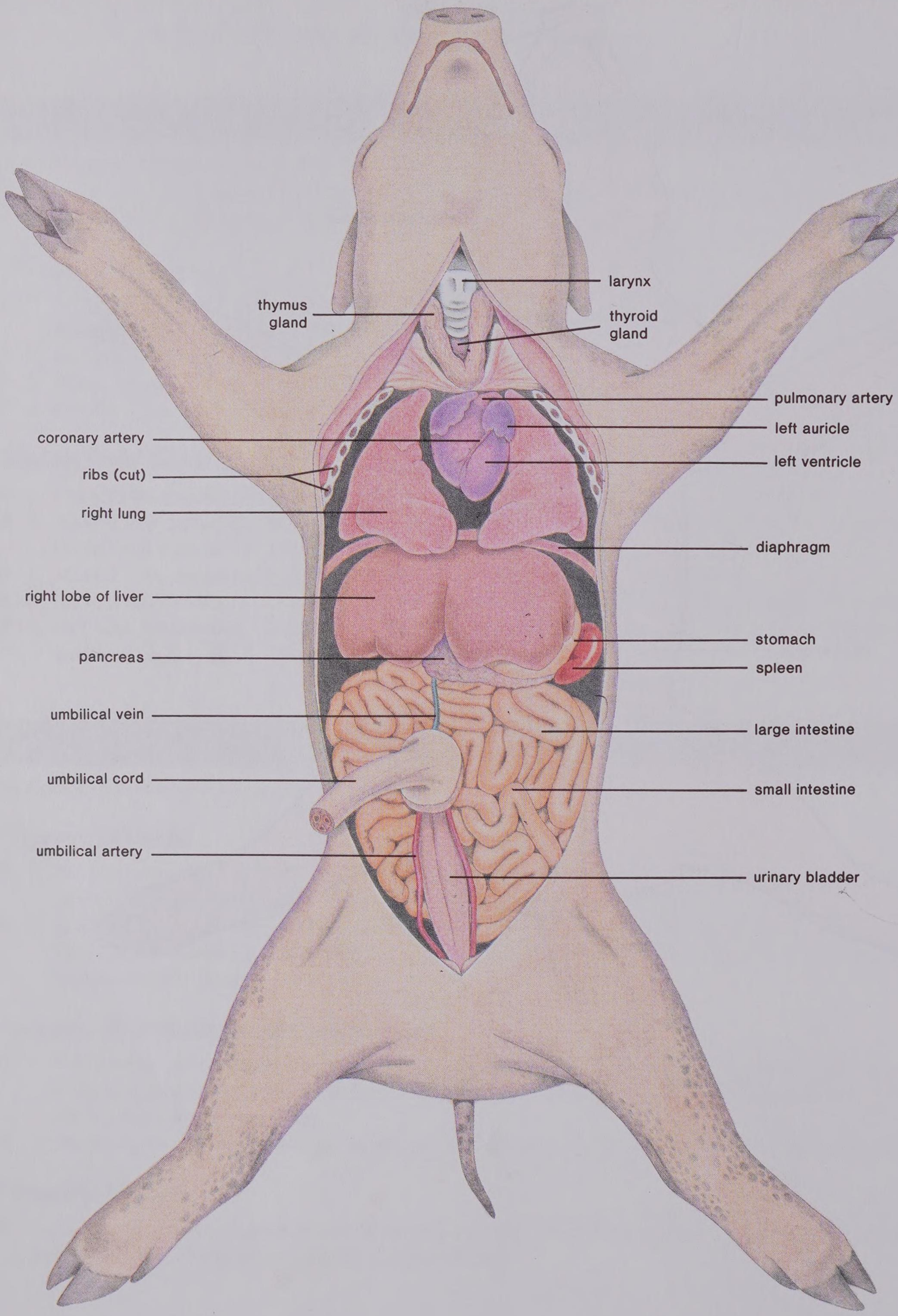


Figure 23.4 Internal anatomy of fetal pig.



Thoracic (Chest) Cavity

Heart and Lungs

S 1. If you have not yet done so, fold back the chest wall flaps. In folding back the flaps, you will need to tear the thin membranes that divide the thoracic cavity into 3 compartments: the left *pleural cavity* containing the left lung, the right pleural cavity, and the *pericardial cavity* containing the heart (fig. 23.5).

S 2. Examine the lungs; locate the 4 lobes of the right lung and the 3 lobes of the left lung. Are the lungs operative or inoperative in the fetus? _____

Dorsal to the heart lies the trachea and its divisions, the bronchi. Later, when the heart is removed, you will have an opportunity to see this division.

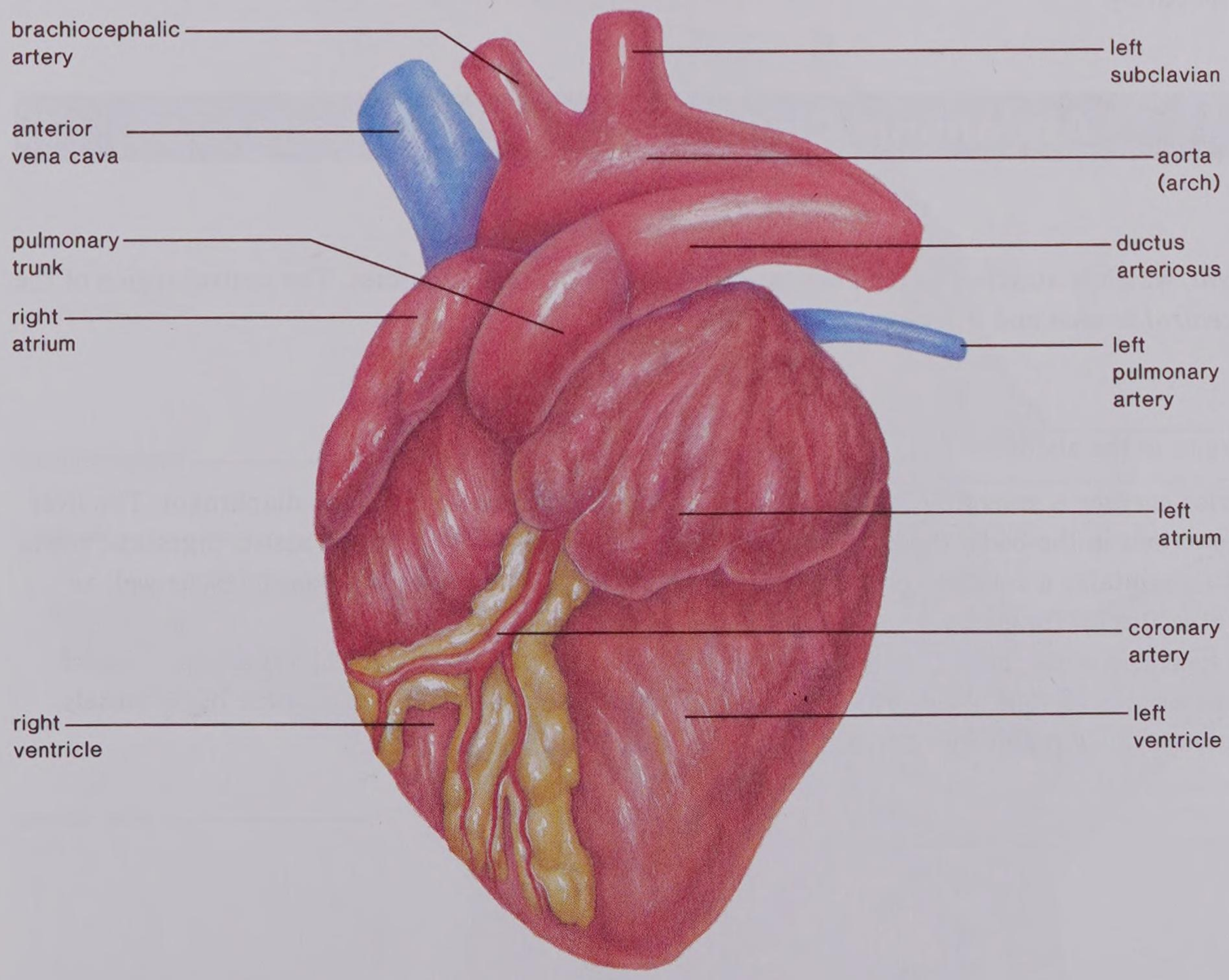
Blood Vessels

Fetal circulation differs from adult circulation in several ways; these features are listed in table 23.2 for your reference as you examine the heart and blood vessels described in this laboratory.

Table 23.2 Unique Features of Fetal Circulation

1. Arterial duct (*ductus arteriosus*): a short, stout vessel leading directly from the pulmonary trunk to the aorta
2. Oval opening (*foramen ovale*): an opening between the atria (auricles)
3. Umbilical vein: returns blood from the placenta into the liver
4. Venous duct (*ductus venosus*): a continuation of the umbilical vein that takes blood to the posterior vena cava
5. Umbilical arteries: take blood from the iliac arteries to the placenta

Figure 23.5 Fetal pig, ventral view of heart.



In the chest cavity, we can locate the blood vessels for the pulmonary system. These vessels take blood to and from the lungs. We can also locate some of the blood vessels for the systemic system. The blood vessels of the systemic system take blood to and from the rest of the body.

Pulmonary System

The *pulmonary trunk* arises from the ventral side of the heart (fig. 23.5). It may appear to be white, but actually the thick wall prevents the color of the red latex from showing through. In your pig, all arteries have been injected with *red latex* and all veins have been injected with *blue latex*.

1. Trace the pulmonary trunk and notice that it seems to connect directly with the *aorta*. This connection is the arterial duct, or *ductus arteriosus* (table 23.2)
2. Upon closer inspection you will find, in addition to this duct, the *pulmonary arteries,* which leave the pulmonary trunk to go to the lungs. The *pulmonary veins* are difficult to locate, and careful dissection is required.
3. If you wish, clean away the membrane dorsal to the heart.
4. Carefully note blue-colored vessels (*pulmonary veins*) that leave the lungs and trace them to the left atrium of the heart.

Coronary Arteries and Cardiac Veins

Look at the *coronary arteries* and *cardiac veins,* which are easily visible on the surface of the heart. The arteries arise from the aorta just as it leaves the heart, and the veins go directly into the right atrium.

Carotid Arteries and Jugular Veins (fig. 23.6)

1. Find the *aorta,* which as it leaves the heart seems to give off 3 branches, the central one of which divides into the *right* and *left common carotid* arteries. The *jugular veins* may be seen alongside the carotid arteries.
2. Trace these veins to the *anterior vena cava*. These arteries and veins serve the neck and head regions.

Subclavian Arteries and Veins

Find the *subclavian arteries* and *veins,* which serve the forelimbs and are easily identifiable proceeding to and away from these structures, respectively.

Abdominal Cavity (fig. 23.7)

Diaphragm

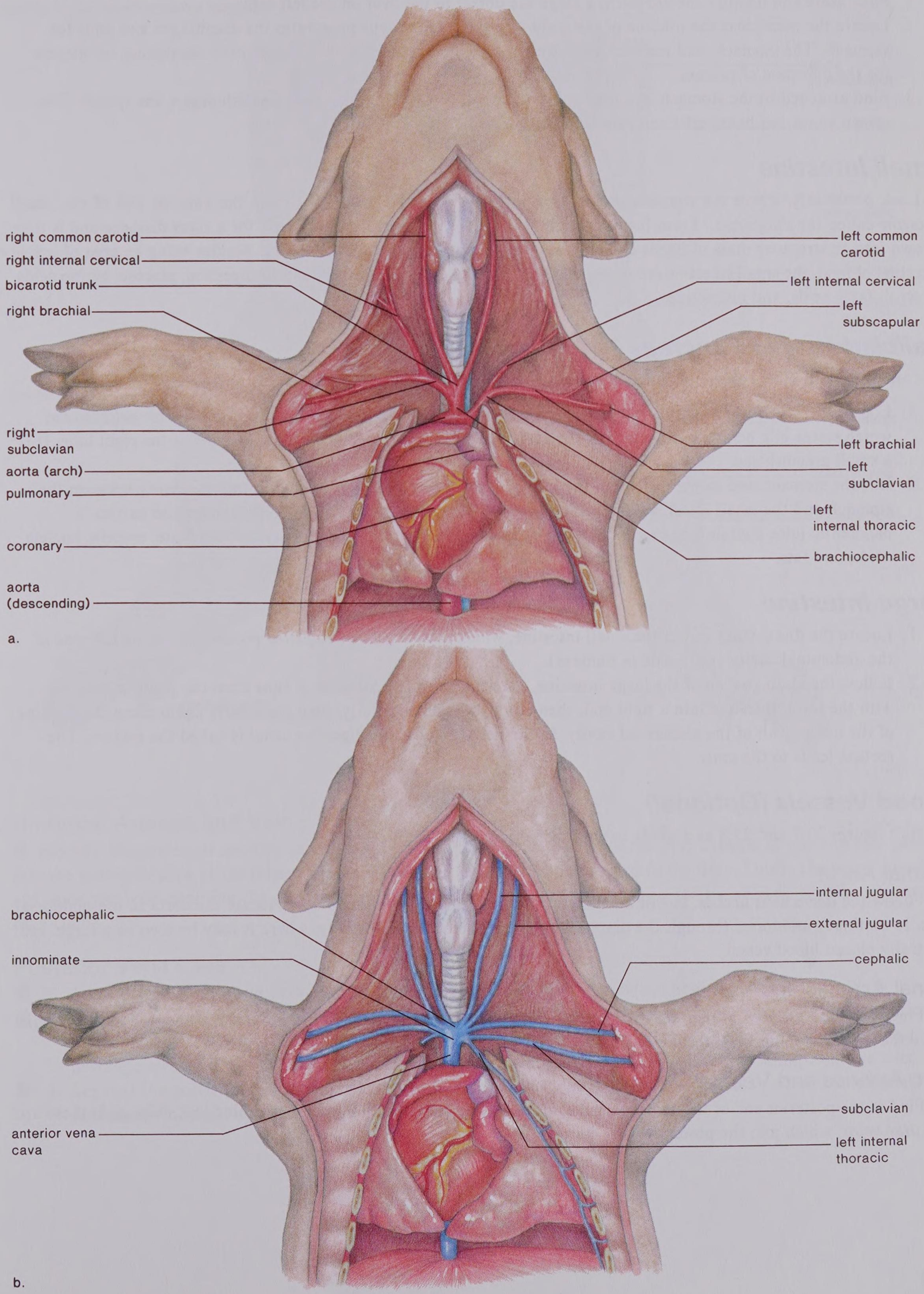
Examine the *diaphragm,* which is attached to the chest wall by radially arranged muscles. The central region of the diaphragm is called the *central tendon* and is a membranous area.

Liver

The *liver* is the largest organ in the abdomen.

1. Note that its anterior *surface* is smoothly convex and fits snugly into the concavity of the diaphragm. The liver serves numerous functions in the body: destroys old red blood cells; produces bile, which assists digestion; stores glucose as glycogen; maintains a constant glucose level in the blood; and produces blood proteins, as well as performing many other useful functions.
2. If your particular specimen is partially filled with dark, brownish material, take your animal to the sink and *rinse* it out. This material is clotted blood, which filled the abdominal cavity when one or more blood vessels burst during injection of the circulatory system.

Figure 23.6 Fetal pig, anterior blood vessels. ***a.*** Anterior arteries. ***b.*** Anterior veins. The bracheocephalic artery branches into the carotid trunk and the right subclavian artery.



Stomach and Spleen

1. Push aside and identify the *stomach,* a large sac dorsal to the liver on the left side.
2. Locate the point near the midline of the body where the *esophagus* penetrates the diaphragm and joins the stomach. The stomach wall contains numerous *gastric glands* that secrete gastric juice containing an enzyme for the digestion of protein.
3. Find attached to the stomach by mesentery (membranous tissue) a long, flat, reddish organ, the *spleen.* The spleen stores red blood cells and produces while blood cells.

Small Intestine

Look posteriorly, where the stomach makes a curve to the right and narrows to join the anterior end of the *small intestine* called the *duodenum.* From the duodenum, the small intestine runs posteriorly for a short distance and is then thrown into an irregular mass of bends and coils held together by a common mesentery. Besides being an area of active digestion of food, the small intestine is the main site for the absorption of the products of digestion; glucose, amino acids, glycerol, fatty acids, and nucleotides.

Gallbladder and Pancreas

Bile and pancreatic juice enter the duodenum and are used in the digestion of food.

1. Locate the *bile duct,* which runs in mesentery stretching between the liver and duodenum. The *gallbladder,* which stores bile before its release, may be found embedded in the liver on the underside of the right lobe. It is a small greenish sac.
2. Lift the stomach and locate the *pancreas,* the light-colored, diffuse gland lying in the mesentery between the stomach and the small intestine. The pancreas has a duct that empties into the duodenum and carries pancreatic juice containing several enzymes: at least one for each type of food—carbohydrate, protein, nucleic acids, and fats.

Large Intestine

1. Locate the distal (far) end of the small intestine, which joins the *large intestine* posteriorly, in the left side of the abdominal cavity (right side in humans).
2. Follow the main portion of the large intestine, known also as the *colon,* as it runs from the point of juncture with the small intestine into a tight coil, then out of the coil anteriorly, then posteriorly again along the midline of the dorsal wall of the abdominal cavity. In the pelvic region, the digestive canal is called the *rectum.* The rectum leads to the *anus.*

Blood Vessels (Optional)

Consult figures 23.7 and 23.8 as a guide to your dissection.

Dorsal Aorta

Follow the *aorta* as it arches. It will be necessary for you to lift aside the lungs to locate the aorta in the middorsal line. It continues middorsal through the diaphragm and in the abdominal cavity, where it may be seen as a large, red, centrally placed blood vessel.

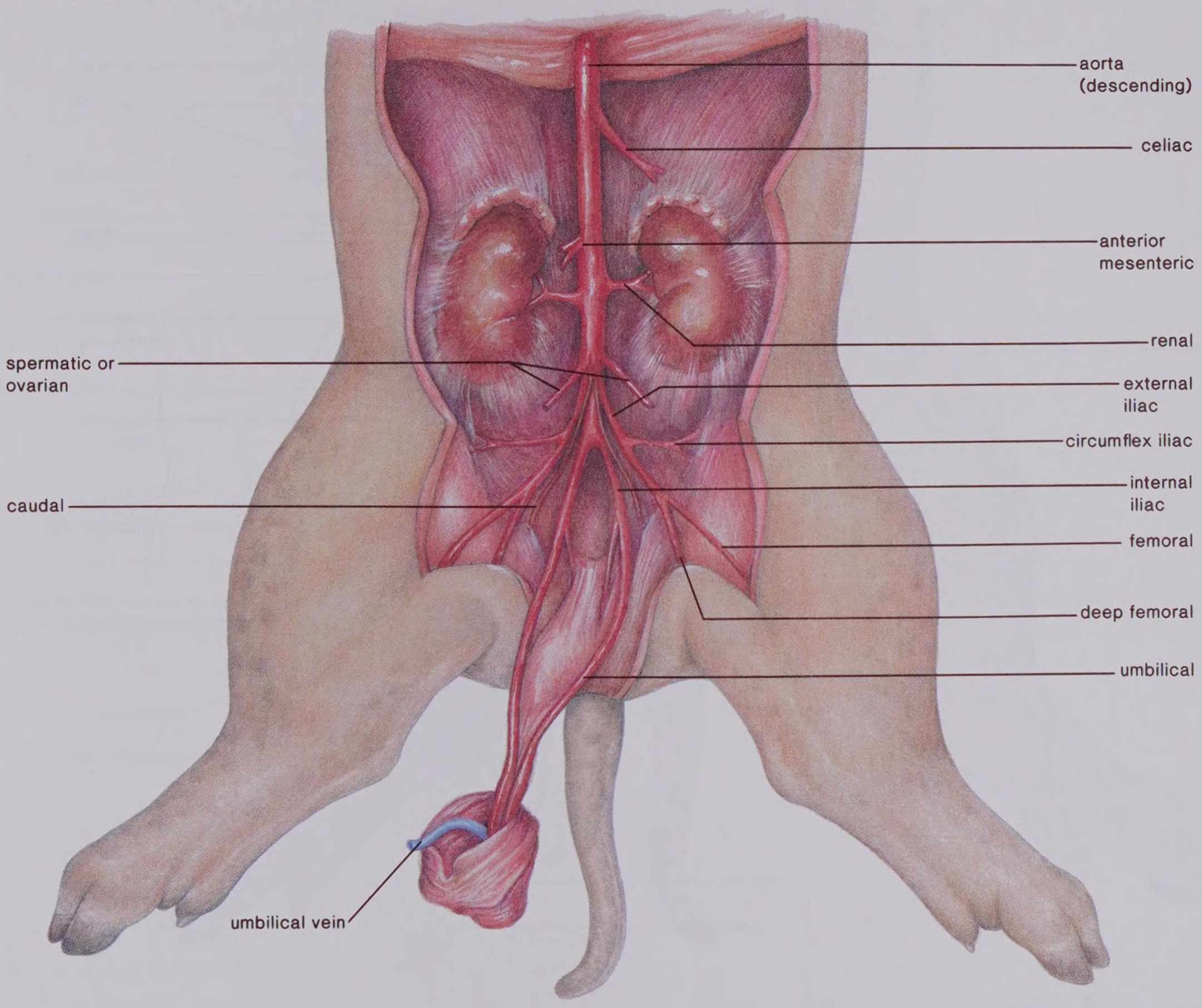
Renal Arteries and Veins

Find the *renal arteries,* which are easily seen as branches from the aorta that go into the kidneys. The *renal veins* leave the kidneys to join the inferior vena cava.

Iliac Arteries and Veins

Find at the posterior end of the aorta, paired arteries called *iliacs,* which branch to the hind legs. Alongside these are the *iliac veins,* which join the posterior vena cava.

Figure 23.7 Fetal pig, posterior arteries. The external iliac arteries proceed into the hindlegs from the caudal end of the dorsal aorta. The internal iliacs also branch from the dorsal aorta. They give rise to the umbilical arteries and continue as much smaller vessels.



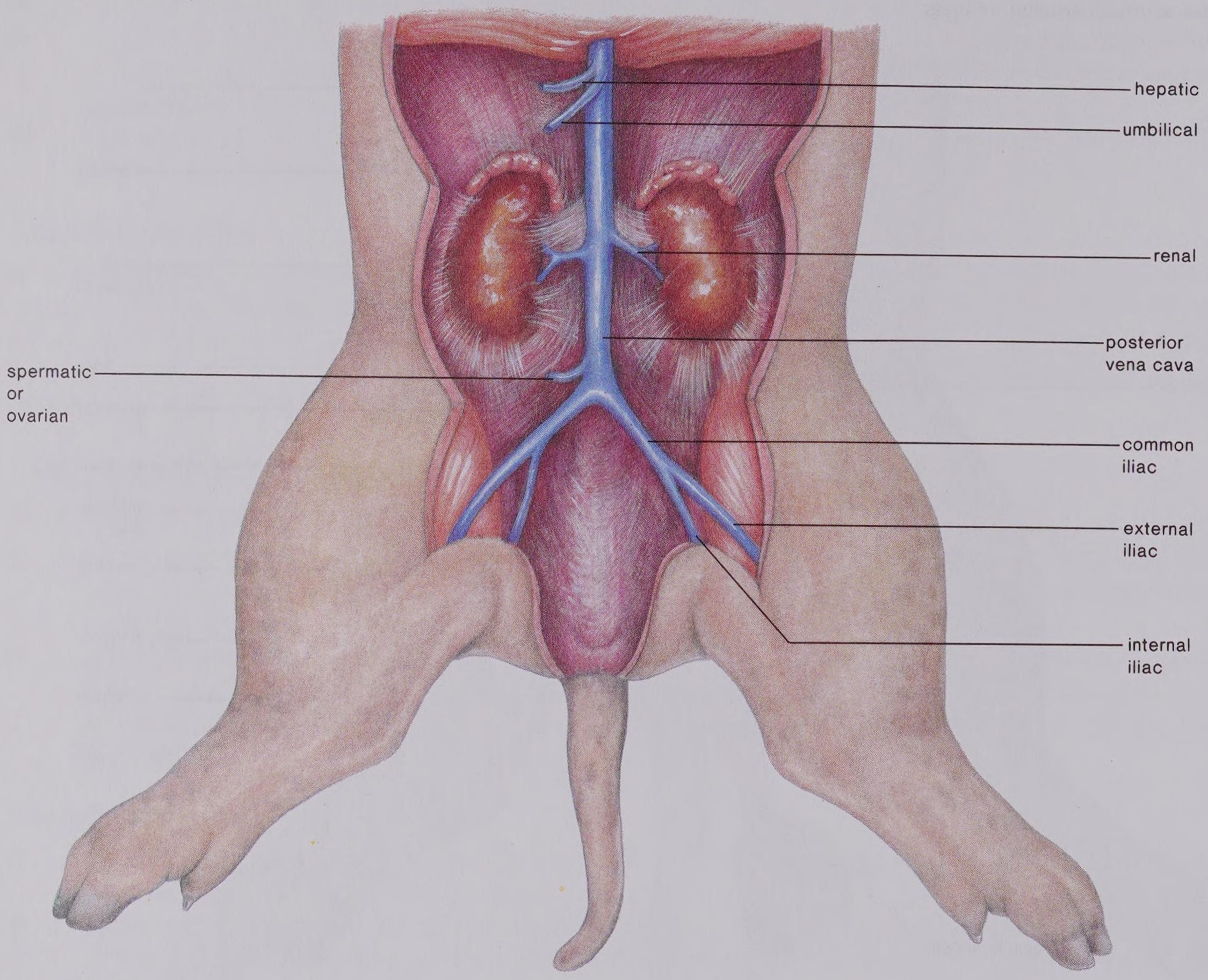
Umbilical Arteries and Vein

Identify the *umbilical arteries* on either side of the bladder, connected to the iliac arteries at one end and passing into the umbilical cord at the other. The *umbilical vein* proceeds from the cord to the liver. This is the vessel that was cut when you were exposing the abdominal cavity to view. The umbilical vein is joined to the vena cava by the venous duct (table 23.2).

Posterior Vena Cava

1. Trace the *posterior vena cava* from the iliac veins to the right atrium of the heart.
2. Find in the abdominal cavity, the *posterior vena cava,* which is easily seen as a large blue vessel just superior to the dorsal aorta; however, it seems to disappear in the region of the liver. To see the vena cava, it is necessary to scrape away some of the liver tissue. Here the vena cava receives the hepatic veins coming from the liver.
3. See that the posterior vena cava is easily found on the thoracic side of the diaphragm as a large blue vessel that enters the right atrium.

Figure 23.8 Fetal pig, posterior veins. The posterior vena cava divides into the common iliac veins, which branch into the external and internal iliac veins.



Storage of Pigs

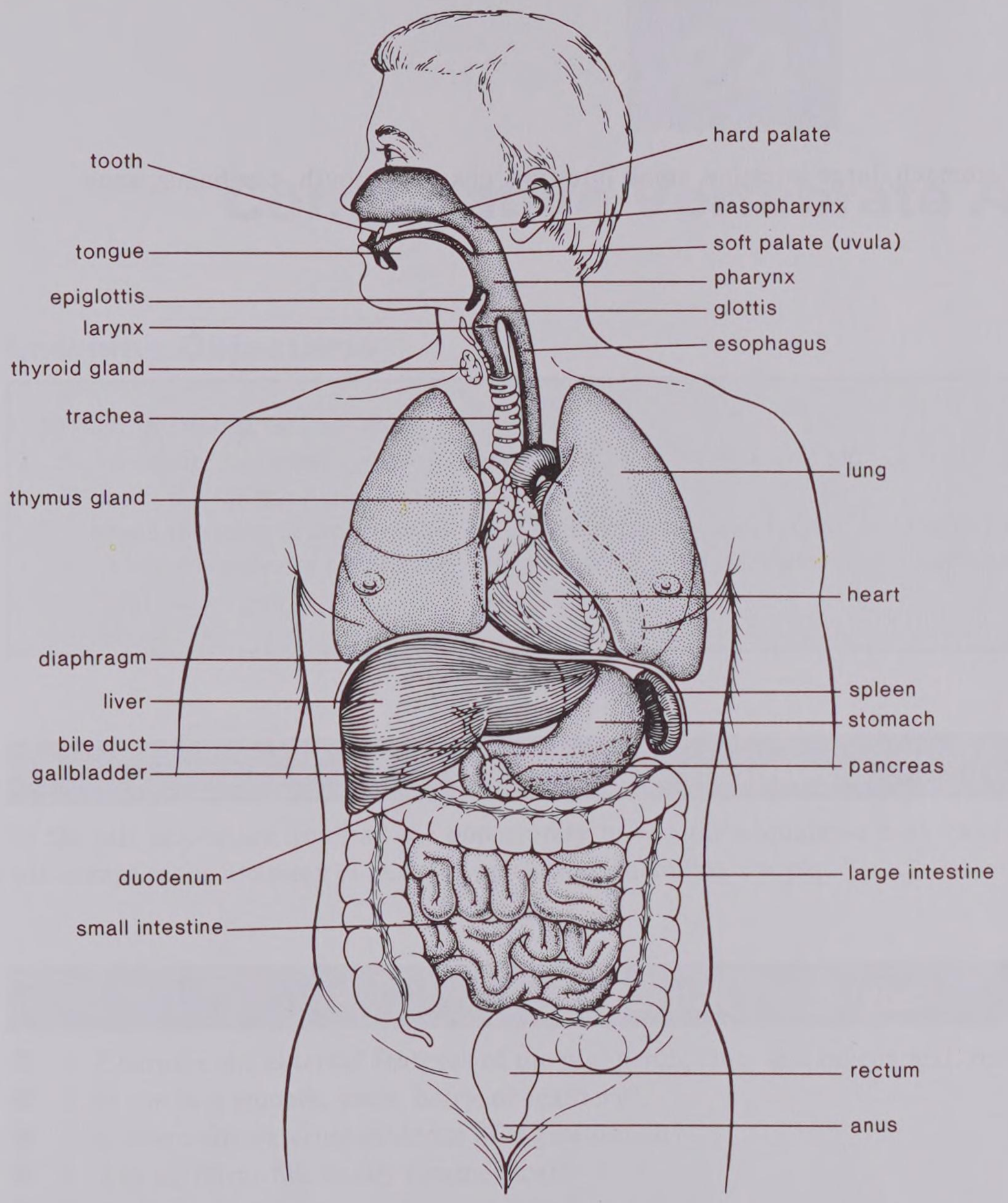
- S 1. Before leaving the laboratory, place your pig in the plastic *bag* provided.
- S 2. Expel excess air from the bag and *tie* it shut.
- S 3. Write your *name* and *section* on the tag provided and attach it to the bag. Your instructor will indicate where these bags are to be stored until the next laboratory period.
- S 4. *Return* the dissecting tray and tools to their proper location.

Human Anatomy

S Examine a human torso model and, using figure 23.9 as a guide, locate the same organs you have just dissected in the pig.

Do you see any major differences between pig internal anatomy and human internal anatomy? _____

Figure 23.9 Internal organs of humans.



Laboratory Review 23

_____ 1. What cavity contains the heart and lungs in humans?

_____ 2. The thyroid lies ventral to what structure?

_____ 3. What does the term *dorsal* mean?

_____ All vertebrates have what structure dorsally placed?

_____ 4. What is the external difference between a male and female pig?

_____ 5. What is the largest organ in the abdominal cavity?

_____ 6. In arteries, what is the direction of blood flow with respect to the heart?

_____ 7. What is the function of the gland that is visible when the stomach is lifted?

_____ 8. How do the lungs in the fetal pig differ from those in the adult?

9. Do arteries always carry oxygenated blood? Explain.

10. Put the following organs in order: stomach, large intestine, small intestine, pharynx, mouth, esophagus, anus.

Laboratory

24

Comparative Vertebrate Anatomy

Learning Objectives

Students should be able to

1. compare the organ systems of the perch, frog, pigeon, and pig;
2. compare human blood to frog blood;
3. trace the path of blood through the heart of a fish, amphibian, and mammal;
4. relate the aspects of the respiratory system to the animal's environment and to features of the circulatory system.

Introduction

In the last laboratory, you had the opportunity to become acquainted with basic mammalian anatomy. This week you will compare the anatomy of certain vertebrates, including the pig.

External Features

S 1. Compare the external features of the pig, perch, frog, and pigeon and, record your observations in table 24.1.
S 2. Is the skin smooth, scaly, hairy, or feathery?
S 3. Is there any external evidence of segmentation?
S 4. Are all forms bilaterally symmetrical?
S 5. Is the body differentiated into regions?
S 6. Is there a well-defined neck?
S 7. Is there a postanal tail?
S 8. Identify the external apertures: are there nares, is there a cloaca, or are the urogenital and anal openings separate?
S 9. Are eyelids present? How many?
S 10. Is there external evidence of an ear?
S 11. How many limbs are there?
S 12. How many digits?
S 13. Are nails or claws present?

The perch, pigeon, and pig have a nearly impenetrable covering.

Why is this an advantage in each case? _____

_____

The frog uses its skin for what purpose not used by the others? _____

Table 24.1 Comparison of External Features

	Perch	Frog	Pigeon	Pig
a. Skin				
b. Segmentation				
c. Symmetry				
d. Regions				
e. Neck				
f. Postanal tail				
g. Nares				
h. Cloaca				
i. Eyelids				
j. External ear				
k. Limbs				
l. Digits in forelimb				
m. Digits in hindlimb				
n. Claws or nails				

Internal Organs

S 1. Examine the *internal organs* of the perch, frog, pigeon, and pig.
S 2. If necessary, make a median longitudinal incision in the ventral body wall from the jaws to the cloaca or anus. The body cavity is a coelom.

Which of these animals have (has) a diaphragm dividing the body cavity into thorax and abdomen? _____

Circulatory System

S 1. If available, study the *heart models* for a fish, amphibian, bird, and mammal.
S 2. Open the heart in the animals you are dissecting and determine how many *atria* and *ventricles* are present.
S 3. Trace the path of the vessel that leaves the ventricle(s) and determine whether the animal has a *pulmonary system* (fig. 24.1). Just behind the heart in the frog can be found a triangular membranous sac, the *sinus venosus*.
S 4. Locate this vessel (*sinus venosus*), which enters the right atrium.
S 5. Complete table 24.2.

Figure 24.1 Comparison of circulatory systems in vertebrates. ***a.*** In a fish, the blood moves in a single loop. The heart has a single atrium and ventricle and serves to pump the blood into the gill region, where gas exchange takes place. Blood pressure created by pumping of the heart is dissipated after the blood passes through the gill capillaries. This is a disadvantage of this single-loop system. ***b.*** Amphibians have a double-loop system in which the heart pumps blood to both the gills and the body itself. The system is not very efficient because oxygenated blood and deoxygenated blood mix in the single ventricle. ***c.*** The pulmonary and systemic systems are completely separate in birds and mammals since the heart is divided by a septum into a right and left half. The right side pumps blood to the lungs and the left side pumps blood to the body proper.

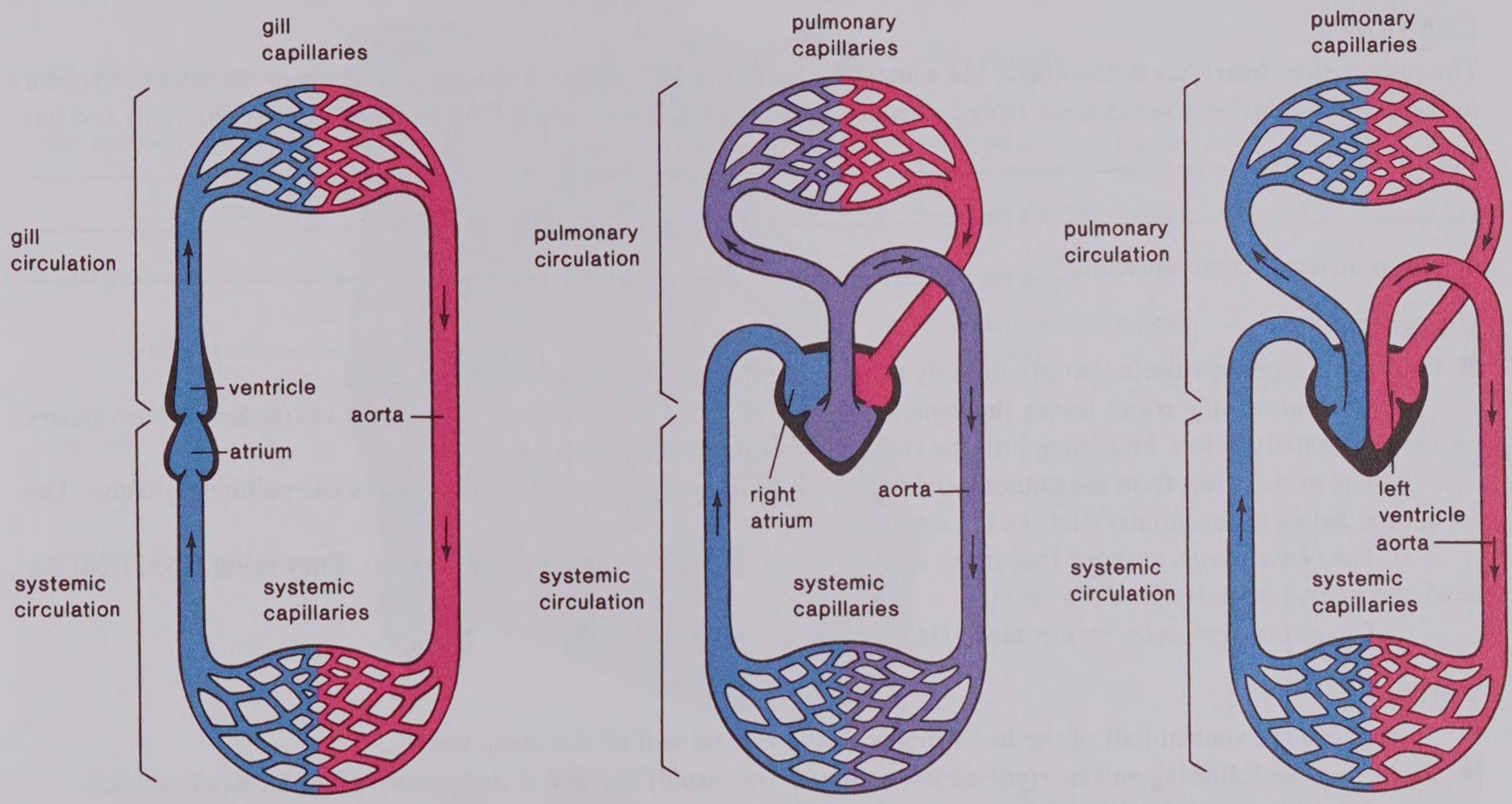


Table 24.2	Comparative Circulatory Systems	
Animal	**Number of Heart Chambers**	**Pulmonary System (yes or no)**
Perch		
Frog		
Pigeon		
Pig		

Do fish have a separate circulatory system to the gills? _____

Would you expect blood pressure to be high or low after blood has moved through the gills? _____

What animals studied have a separate circulatory system for the respiratory organ? _____

What is the advantage of having a separate circulatory system that returns blood to the heart? _____

_____

Which of these also have (has) a 4-chambered heart? _____

What is the advantage of having separate ventricles? _____

_____

Contrast the body temperature of those animals with a 4-chambered heart to those that do not have a 4-chambered heart.

Comment. _____

_____

Mammalian Heart

Identify the following structures by referring to a *heart model* or preserved *sheep heart* (see also fig. 24.2).

Chambers

The mammalian heart has 4 chambers: the *ventricles,* right and left, compose the major portion of the heart. Anterior to these are the darker colored *atria* (sing., *atrium*), sometimes called auricles. How can you identify the right and left sides of the heart? _____

_____

Which ventricle is more muscular? _____

Vessels

Find the 4 types of vessels that are directly connected to the heart in mammals (fig. 24.2).

The *pulmonary trunk* leaves the ventral side of the heart from the top of the right ventricle and then passes forward diagonally before branching into the right and left pulmonary arteries.

The *aorta* arises from the anterior end of the left ventricle, just dorsal to the origin of the pulmonary trunk. The aorta soon bends to the animal's left as the aortic arch.

The *venae cavae,* anterior (superior) and posterior (inferior), enter the right atrium. They bring blood from the head and body, respectively, to the heart.

The *pulmonary veins* return blood from the lungs to the left atrium.

Valves

1. Remove the ventral half of the heart model or the ventral half of the sheep heart.
2. Locate the following valves: *right atrioventricular* (tricuspid) valve; *left atrioventricular* (bicuspid, mitral) valve; *pulmonary semilunar* valve (in the base of the pulmonary trunk); *aortic semilunar* valve (in the base of the aorta).
3. Note the "heartstrings" (chordae tendineae) that hold the valves in place while the heart contracts.

Nodes

1. Identify the *SA* (sinoatrial) *node,* also called the *pacemaker,* in the wall of the right atrium. This node initiates the heartbeat. Why is the sinoatrial node of mammals so called? _____

_____

_____

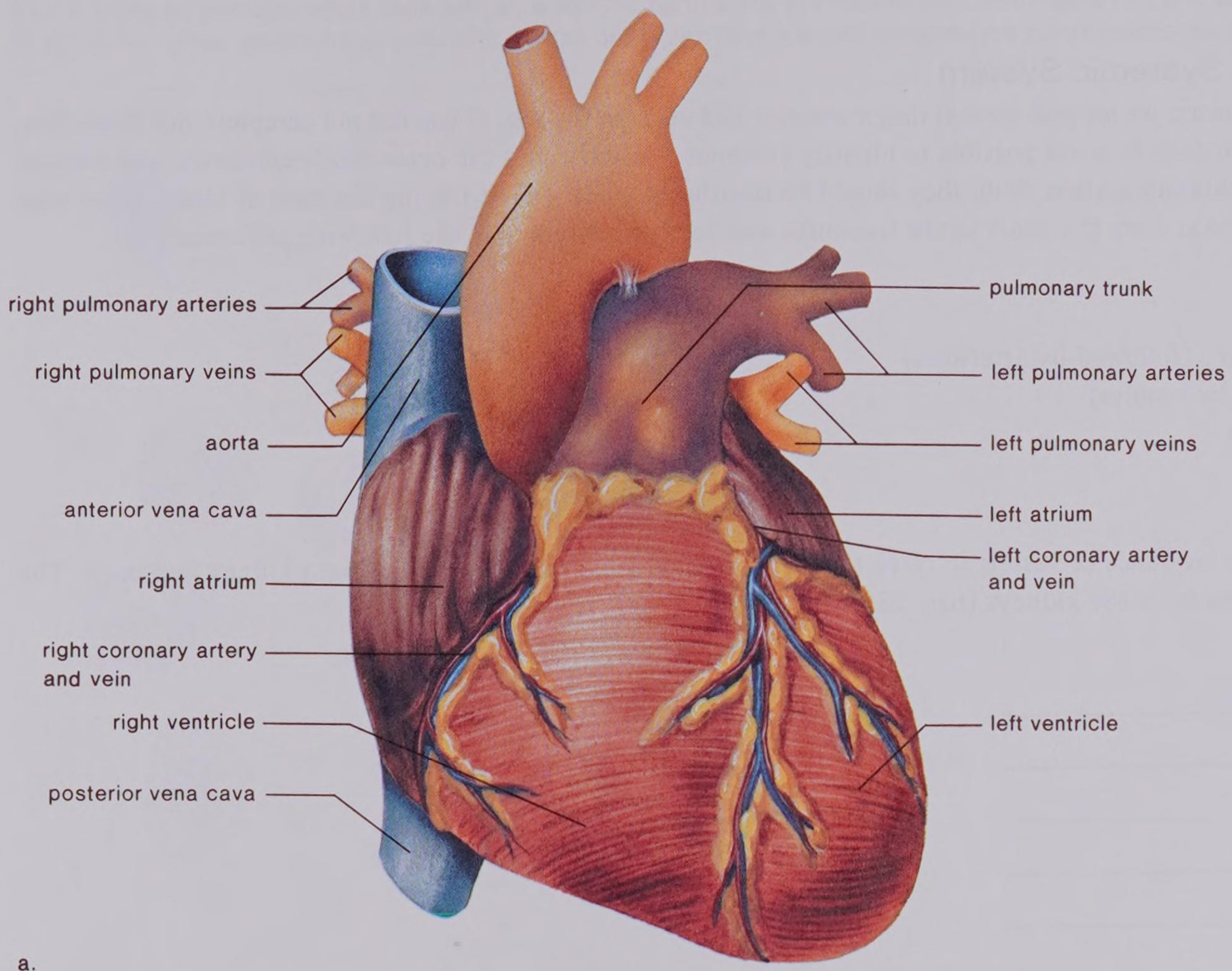
2. Identify the *AV* (atrioventricular) *node* in the septum wall. This node receives signals from the pacemaker and transmits impulses to the ventricles.

Path of Blood through Mammalian Heart

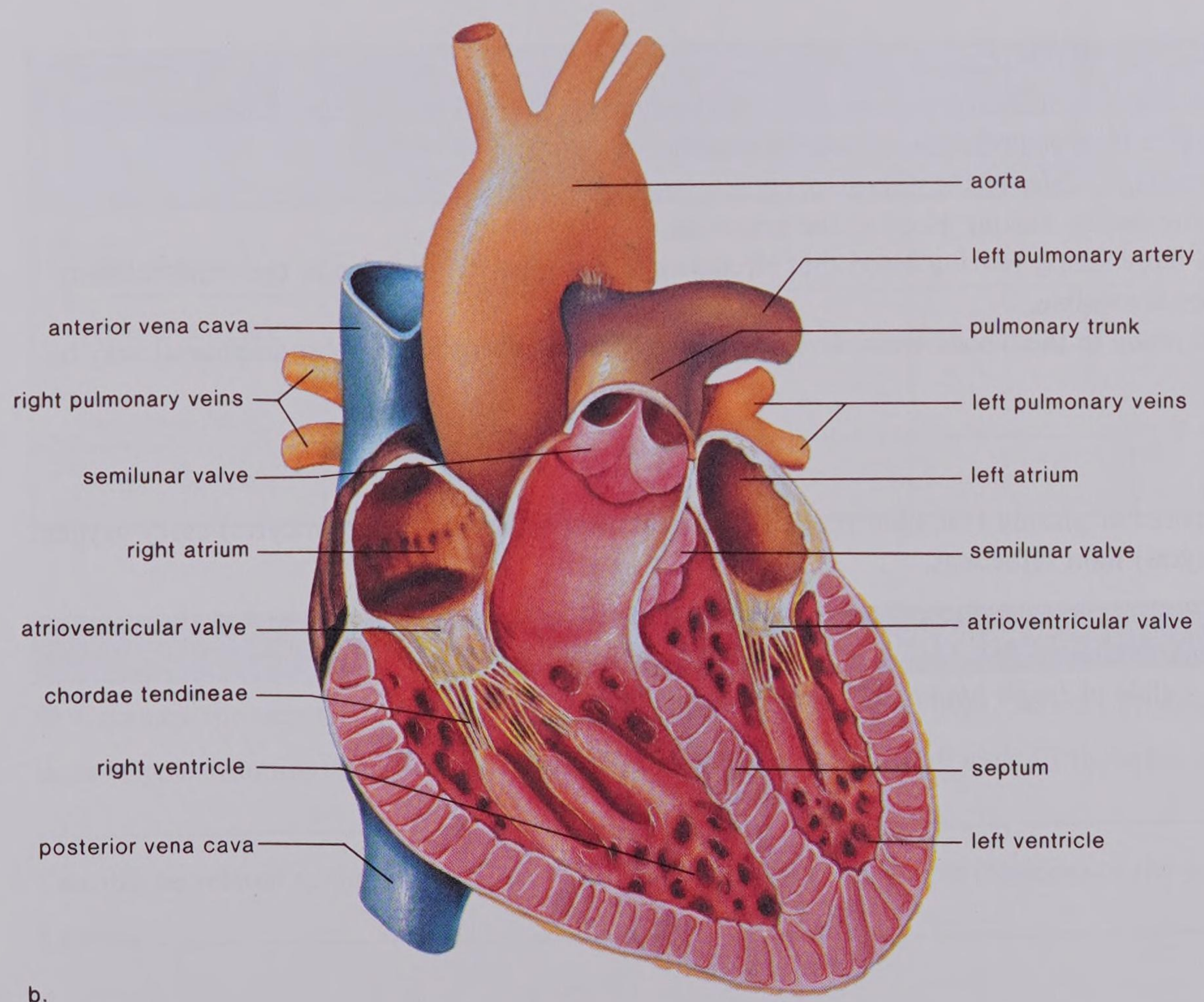
1. Trace the path of blood from the right side of the heart to the aorta by filling in the blanks that follow.
2. Notice that in the adult mammal the blood passes through the lungs to go from the right to the left.

Venae Cavae	**From Lungs**
_____ (chamber)	_____ (vessel)
_____ (valve)	_____ (chamber)
_____ (chamber)	_____ (valve)
_____ (valve)	_____ (chamber)
_____ (vessel)	_____ (valve)
To Lungs	Aorta

Figure 24.2 Human heart. ***a.*** External view. ***b.*** Internal view.



a.



b.

Ⓢ 3. With the help of figure 24.1, compare the path of blood through the mammalian heart with that of the other animals.

Path of Blood in Systemic System

In the previous laboratory, we located several major arteries and veins in the pig. If you did not complete this dissection, you may wish to do so now. It is *not* possible to identify (without a microscope) the *arterioles, capillaries,* and *venules* of the systematic circulatory system. Still, they should be mentioned when we are tracing the path of blood. If we wish to trace the path of blood from the heart to the forelimbs and back, we might give the following sequence:

Left ventricle
Aorta
Right subclavian artery (followed by arterioles)
Capillaries (followed by venules)
Right subclavian vein
Superior vena cava
Right atrium

Ⓢ Provide the proper sequence of vessels to trace the path of blood from the heart to the right kidney and back. The renal arteries and veins serve the kidneys (figs. 23.7 and 23.8).

Left ventricle

_____

_____

_____

_____

_____

Right atrium

Blood Flow (Demonstration)

Ⓓ 1. Observe blood flow through capillaries, arterioles, and venules either in the tail of a goldfish or in the webbed skin between the toes of a frog, as prepared by your instructor.
Ⓢ 2. Examine under low and high power of the microscope.
Ⓢ 3. Watch the *pulse* and the swiftly moving blood in the arterioles.
Ⓢ 4. Contrast this with the more slowly moving blood that circulates in the opposite direction in the venules. Many crisscrossing capillaries are visible.
Ⓢ 5. Look for *blood cells* floating in the bloodstream. Irregular black patches of pigment (chromatophores) may be visible in the skin.

Blood Cells

Blood is a liquid tissue, composed of plasma (the fluid portion) and cells. Red blood cells (erythrocytes) carry oxygen, and white blood cells (leukocytes) fight infections.

Ⓢ 1. Study a prepared slide of *human blood* (fig. 24.3), and note the disc-shaped *red* blood cells and the less numerous and larger *white* blood cells.
Ⓢ 2. Compare this slide to a slide of *frog's blood* and complete table 24.3.

Figure 24.3 A representation of human blood cells. Shown are red blood cells whose scientific name is erythrocytes (Er); 3 kinds of granular white cells whose scientific names are eosinophil (EO), neutrophil (N), and basophil (B); 2 kinds of agranular white cells whose scientific names are monocyte (M) and lymphocyte (L); platelets are also shown (P).

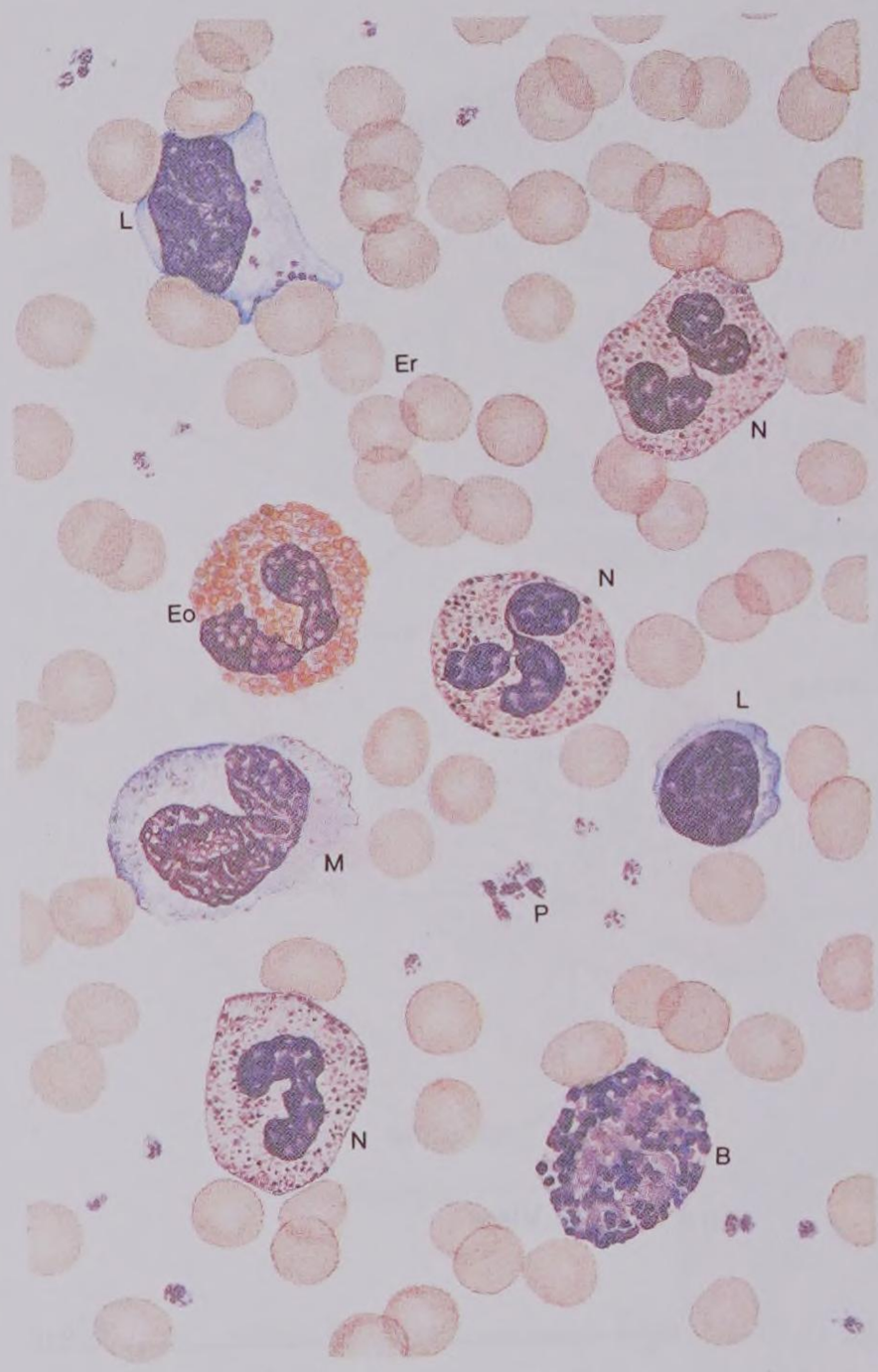


Table 24.3 Comparison of Human Blood with Frog Blood

	Relative Number	Relative Size	Presence of Nucleus	Function
Human Red blood cells				
White blood cells				
Frog Red blood cells				
White blood cells				

Respiratory System

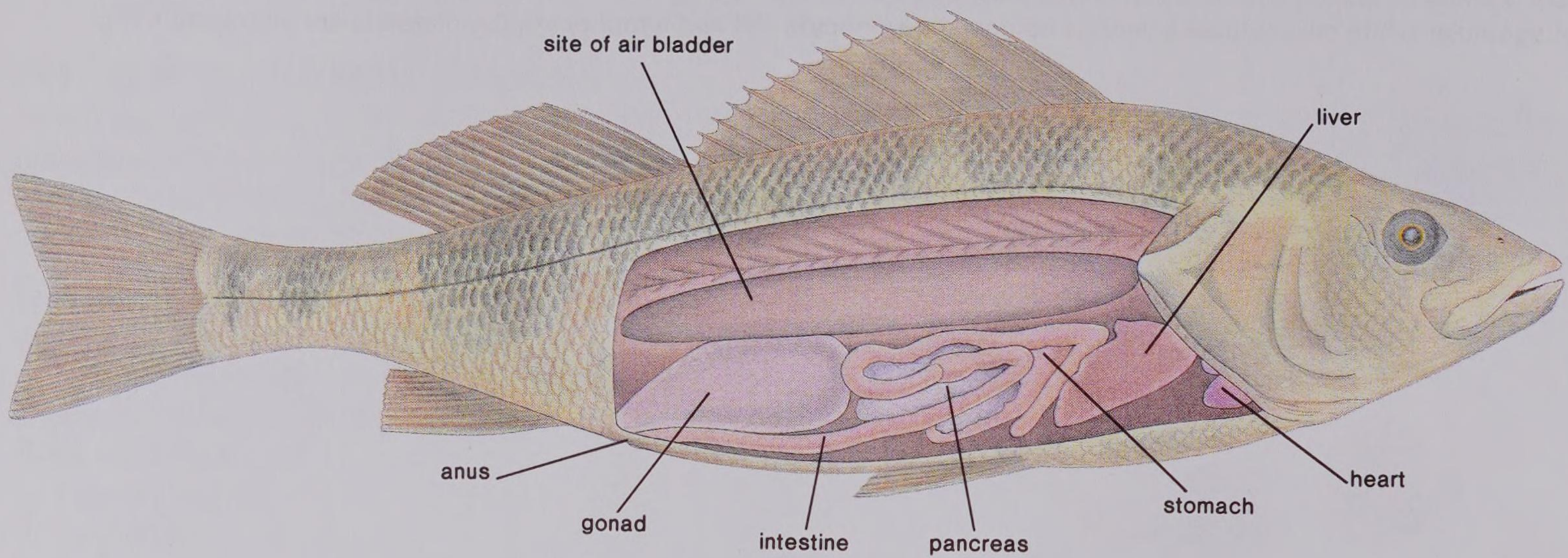
Compare the respiratory systems of the animals you are studying (figs. 24.4–24.7), and complete table 24.4. On the basis of your examination, contrast the respiratory system of the perch with all the other animals. _____

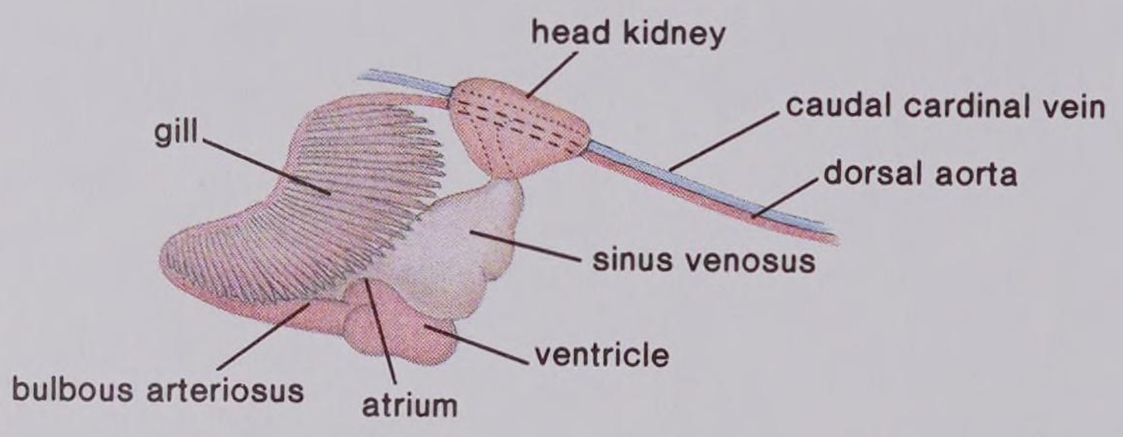
_____

Can this be related to the environment of the perch compared to the environment of the other animals? _____

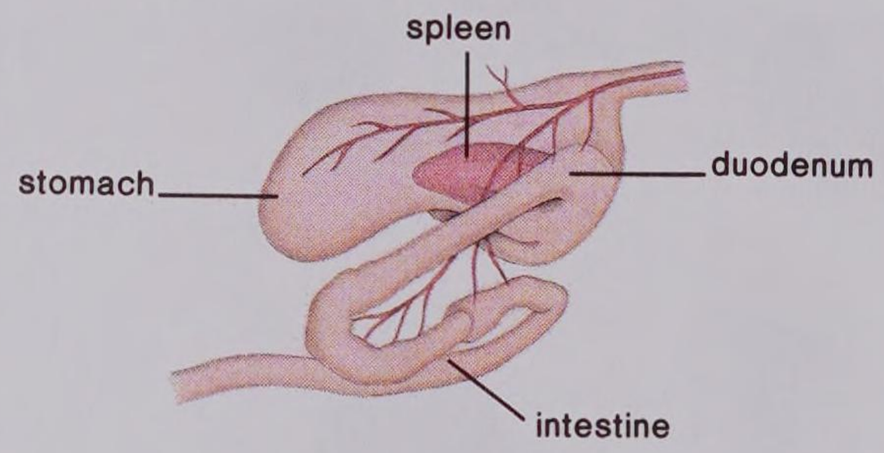
Explain. _____

Figure 24.4 Perch anatomy.





Left Lateral View



Right Lateral View

What anatomical feature present in reptiles and mammals is missing in the frog? _____ Can this be related to the fact that frogs breathe by positive pressure, while birds and mammals breathe by negative pressure? (A frog swallows air and pushes it into its lungs; in birds and mammals the chest expands first, and then the air is drawn in.) Explain. _____

What anatomical feature is present only in birds? _____ This feature allows the air to pass one way through the lungs of a bird and greatly increases their ability to extract oxygen from air. What anatomical feature is present only in mammals? _____ Of what benefit is this feature to mammals? _____

_____

Table 24.4 Respiratory Systems

	Glottis	Larynx	Trachea	Lungs	Rib Cage	Diaphragm	Air Sacs
Perch							
Frog							
Pigeon							
Pig							

Figure 24.5 Frog, internal anatomy.

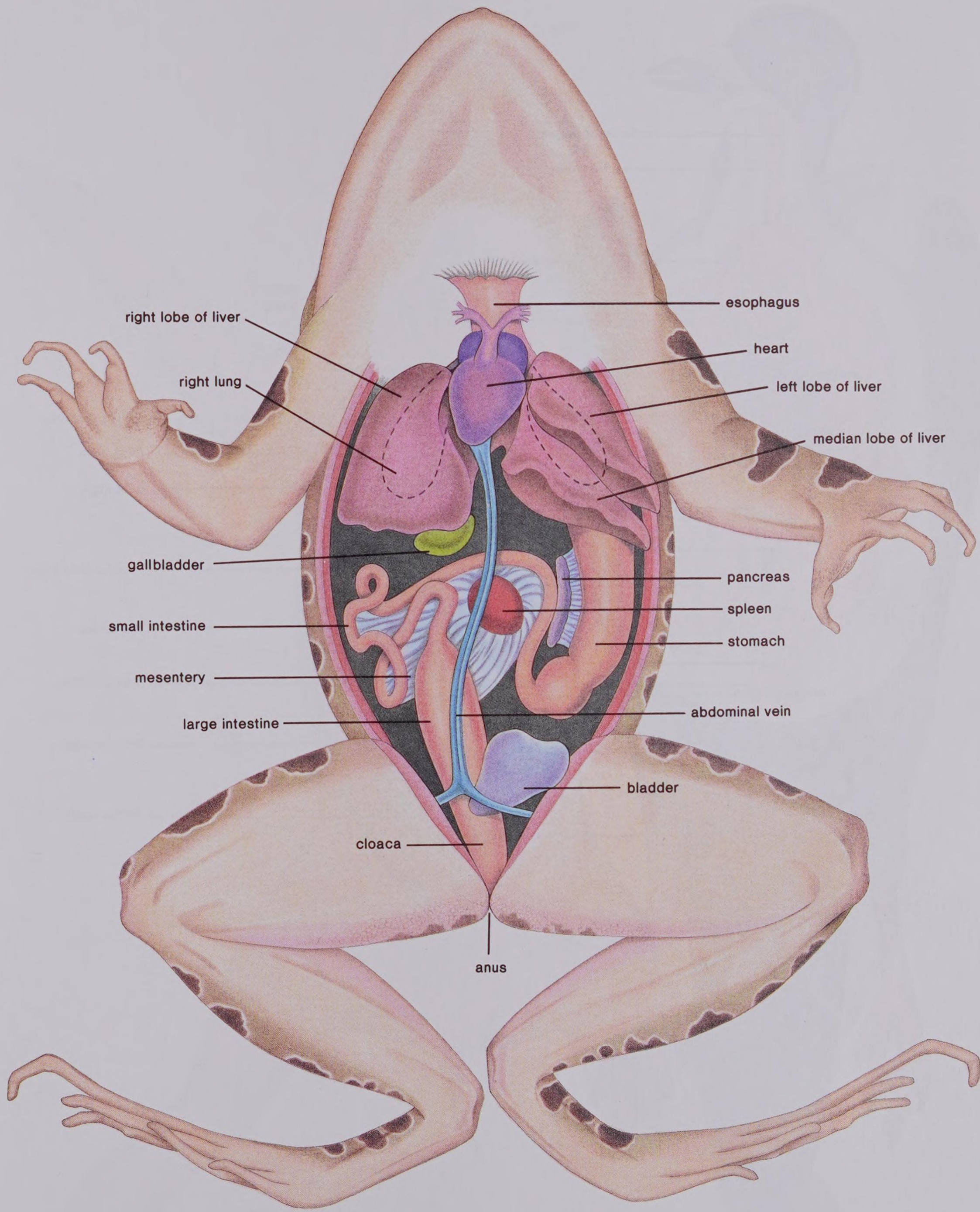


Figure 24.6 Pigeon anatomy.

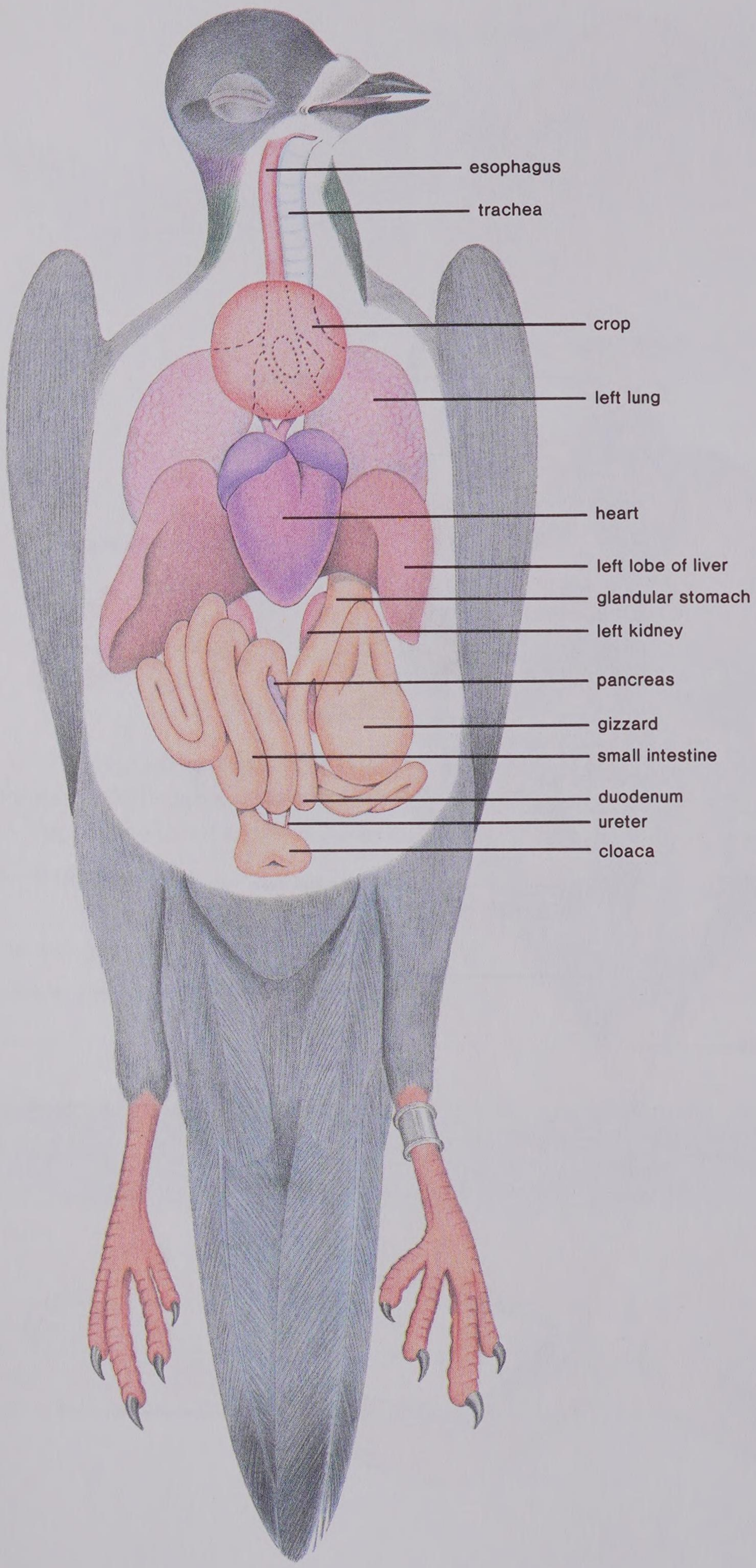
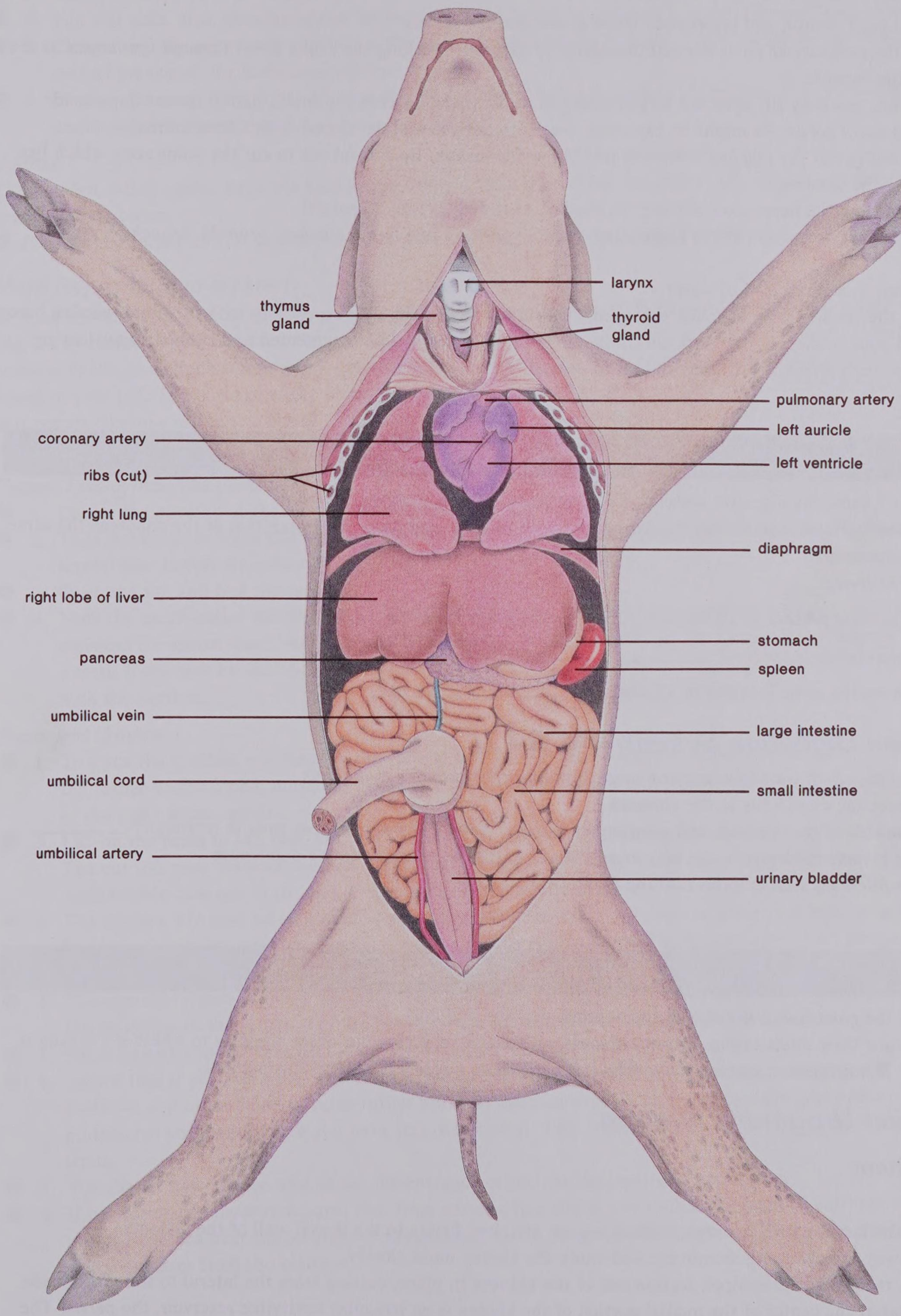


Figure 24.7 Internal anatomy of fetal pig.



Mammalian Respiratory System

1. Open the pig's mouth, and insert your blunt probe into the *glottis*.
2. Explore the pathway of air in normal breathing by carefully working the probe down through the *larynx* to the level of the *bronchi*.
3. If you wish, you may slit open the larynx along its midline and observe the small, paired lateral flaps inside known as *vocal cords*. As might be expected, these are not yet well developed in this fetal animal.
4. Very carefully, cut the *trachea* crosswise just below the larynx. Be careful not to cut the esophagus, which lies just below the trachea.
5. After removing the heart, you will see the trachea as it divides into 2 *bronchi*.
6. Lift out the entire portion of the respiratory system you have just freed: *trachea, bronchi, bronchioles,* and *lungs*.
7. Place them in a small dish of *water*.
8. Holding the trachea with your forceps, gently but firmly stroke the lung repeatedly with the blunt wooden base of one of your probes. If you work carefully, the alveolar tissue will be fragmented and rubbed away, leaving the branching system of air tubes and blood vessels.

Digestive System

Figures 24.4–24.7 show the digestive systems of the animals you are studying.

1. Locate the *stomach, small intestine, large intestine, rectum,* and *anus*. Is the position of the pancreas the same in all specimens?
2. Locate the *liver*.

   Is a gallbladder present in all forms? _____

   Is the spleen always present? _____

   Does it have the same location in all cases? _____

Mammalian Digestive System (fig. 24.7)

1. Open the mouth of the pig again and insert a blunt probe into the *esophagus*.
2. Then, trace the esophagus to the *stomach*.
3. Open one side of the stomach and examine its *interior surface*. Does it appear smooth or rough? _____
4. Find the *pyloric sphincter,* a circular muscle that guards the entrance to the duodenum.
5. Find the *bile duct* that empties into the duodenum.

Urogenital System

1. Identify the *gonads* and *kidneys* in the animals you are studying.
2. Try to trace their ducts to the *urogenital sinus,* and check your previous observation as to whether a cloaca is present. The urogenital system of the frog is described on pages 229–230 of this manual.

Mammalian Urogenital System

Urinary System

Kidneys

1. Locate the large, paired *kidneys,* reddish organs attached firmly to the dorsal wall of the abdomen.
2. *Clean* away the overlying membrane and study the kidney more closely.
3. Using a razor blade or scalpel, *section* one of the kidneys in place, cutting from the lateral to the medial side.
4. Note that at the center of the medial portion of the kidney is an irregular cavitylike reservoir, the *pelvis*. The outermost portion of the kidney (the *cortex*) shows many small striations perpendicular to the outer surface. This region and the more even-textured *medulla* region inward from it are composed of excretory tubules.

Ureters and Bladder

1. Find the midpoint of the medial side of the kidney, a depression where the *renal artery* and *vein* are attached.
2. You will note, also, that the *ureter* leaves the kidney and runs posteriorly under the overlying membrane.
3. Clean away the membrane and follow the ureter to the *urinary bladder,* which normally lies in the posterior ventral portion of the abdominal cavity.
4. In your specimen the urinary bladder will be found on the inner surface of the flap of tissue to which the umbilical cord was attached.

Urethra

The *urethra,* which arises from the bladder posteriorly, runs posteriorly parallel to the rectum. In the adult, it carries urine to the exterior.

- *Follow* the urethra until it passes from view into the ring formed by the pelvic girdle.

Male Reproductive System

Find the male sex glands, the *testes*. They begin their development in the body cavity, just posterior to the kidneys (fig. 24.8). Before birth, however, they gradually descend into paired *scrotal sacs,* which appear as outpouchings just anterior to the anus. Each scrotal sac is connected to the body cavity by an *inguinal canal,* the opening of which may be found in your pig. The passage of the testes from the body cavity into the scrotal sacs is called the descent of the testes and occurs likewise in human males. The testes in most of the male pigs in class will probably be partially or fully descended.

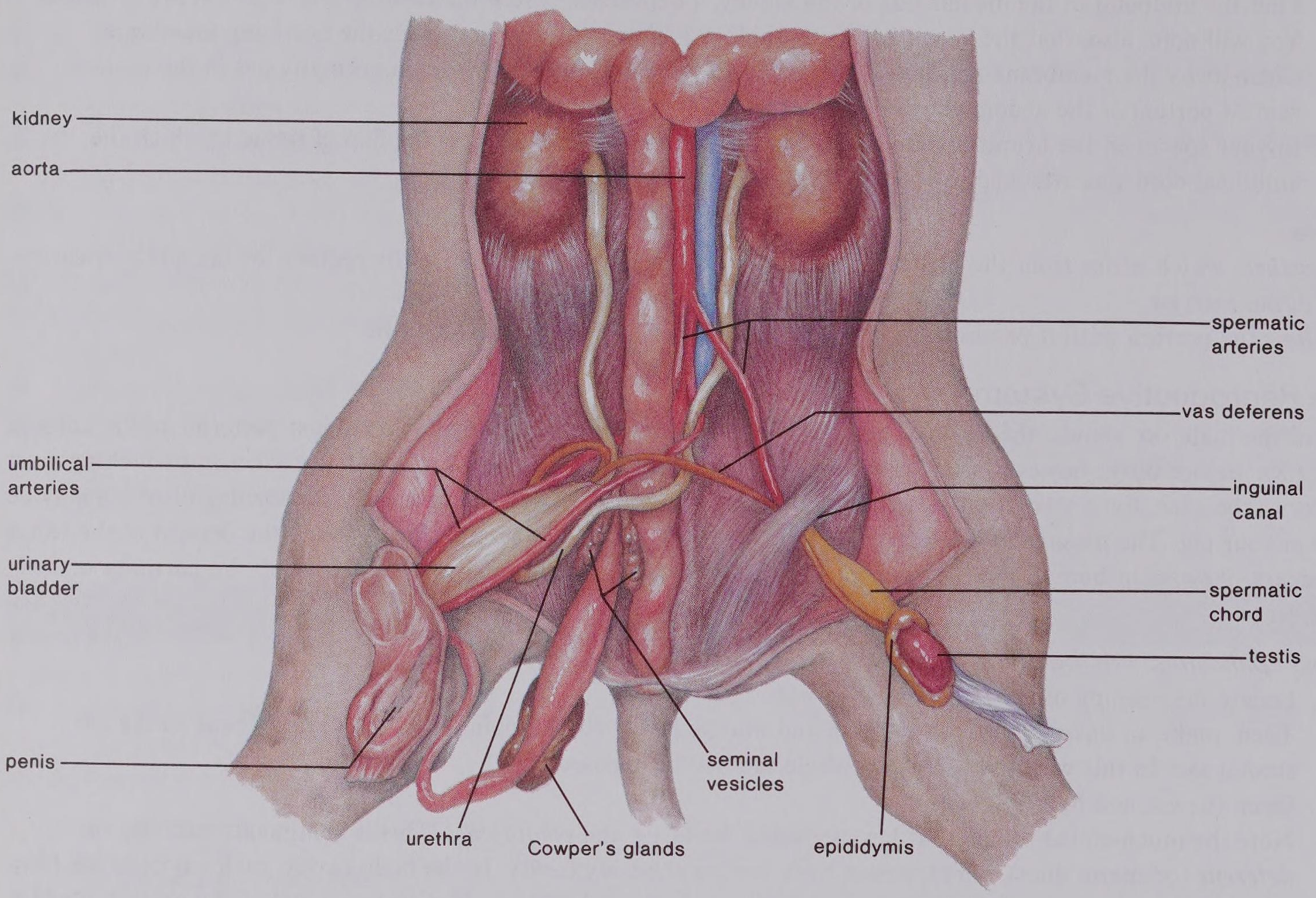
Testes, Epididymis, Vas Deferens

1. Locate the opening of the left *inguinal canal.*
2. Then, make an *incision* through the skin and muscle layers from a point over this opening back to the left scrotal sac. In this way, the canal and whole sac will be exposed.
3. Open the sac and find the *testis.*
4. Note the much-coiled tubule that lies alongside the testis, the *epididymis*. This is continuous with the *vas deferens* (or sperm duct), which passes back toward the body cavity. In the body cavity, each vas deferens from a testis loops over an umbilical artery and ureter and unites dorsally at the posterior end of the urinary bladder with the urethra.

Penis and Urethra

1. To trace the urethra, cut through the ventral skin surface just posterior to the umbilical cord. This will expose the rather undeveloped, muscular *penis,* which extends from this point back toward the anus. The central duct of the penis is the *urethra.*
2. Laying the penis to one side, *cut* down through the ventral midline, laying the legs wide apart in the process. The cut will pass between muscles and through pelvic "cartilage," since bone has not developed. Take considerable care not to cut any of the ducts or tracts in the region.
3. The urethra will now be seen passing ventrally above the rectum. It will be somewhat heavier in the male because of certain accessory glands. *Cowper's glands* (bulbourethral glands; about 1.5 cm long and about 4 mm thick) lie laterally and well back toward the anal opening.
4. The *prostate gland* (about 8 mm long and about 6 mm wide) is located on the dorsal surface of the urethra just posterior to the juncture of the bladder with the urethra; it is often difficult to locate.
5. Small paired *seminal vesicles* may also be seen on either side of the prostate gland.
6. Notice that if you *trace* the urethra as it leaves the bladder, it proceeds posteriorly; but when it nears the posterior end of the body, it turns rather abruptly anterioventrally and runs forward just under the skin of the midventral body wall where you have just dissected it. This latter portion of the urethra is, then, within the penis.
7. You should now also be able to see the entrance of the *vas deferens* into the urethra.
8. If necessary, cut these structures free from surrounding tissue and expose the point of entrance of these ducts into the urethra near the location of the prostate gland. In males, the urethra transports sperm, as well as the urinary wastes from the bladder.

Figure 24.8 Fetal pig, male urogenital system.



Female Reproductive System (fig. 24.9)

Ovaries and Oviducts

1. Locate the paired female gonads, the *ovaries,* small bodies suspended from the peritoneal wall in mesenteries, posterior to the kidneys.
2. Examine one *ovary* closely and note the small, short, coiled *oviduct,* sometimes called the fallopian tube. The oviduct does not attach directly to the ovary but ends in a funnel-shaped structure that partially encloses the ovary. When ovulation takes place, the eggs are carried by ciliary currents into the mouth of the funnel and pass down the oviduct to the uterus.

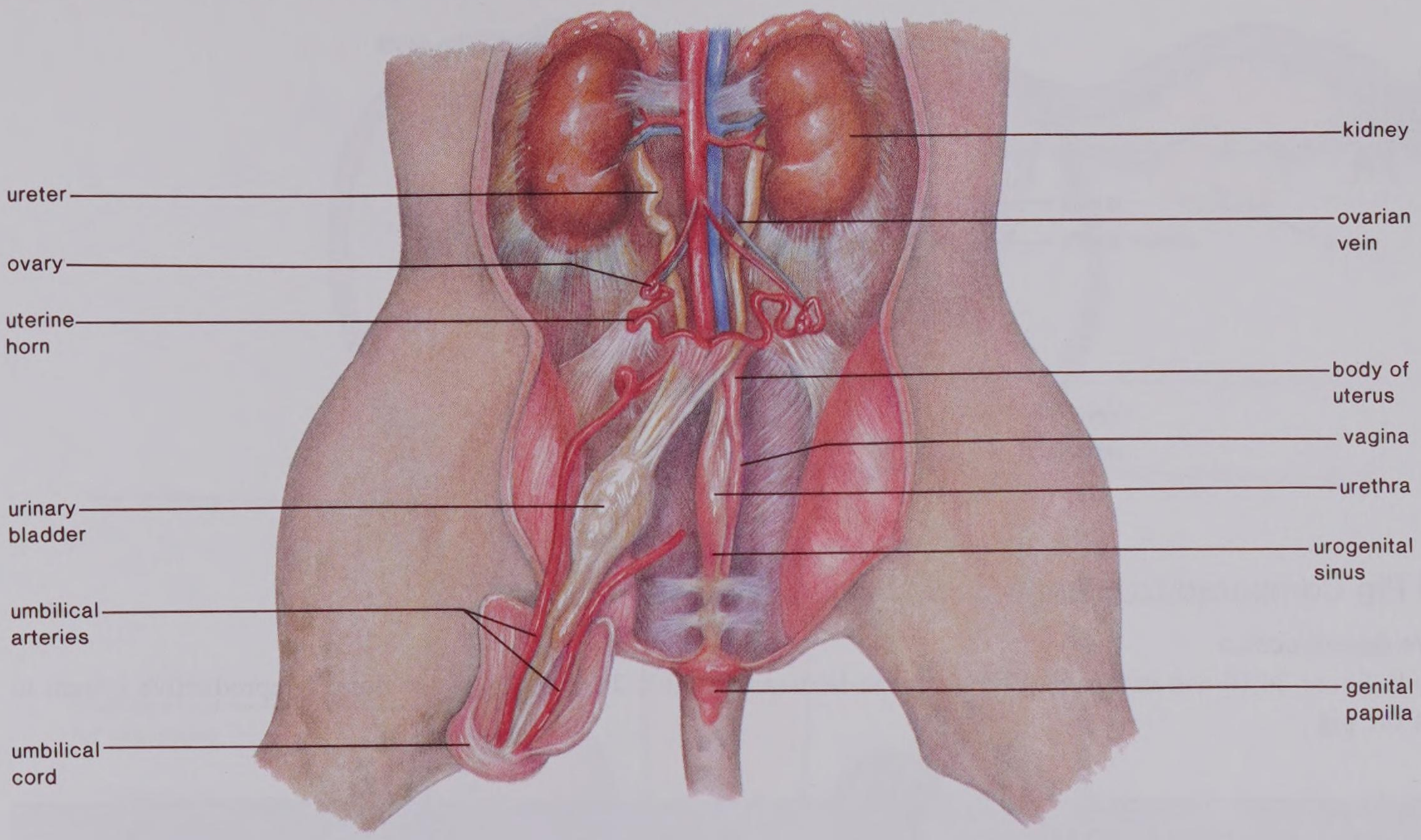
Uterine Horns

1. Locate in your pig the *uterus,* or womb. It is divided into 2 sections called *uterine horns.* At its anterior end, each of the uterine horns is continuous with an *oviduct*; at their posterior ends, the uterine horns join to form the smaller, median body of the uterus.
2. Be careful not to confuse the uterine horns with the oviducts; the latter are much smaller and are located very close to the ovaries.

Vagina

1. To trace the remainder of the female pig reproductive tract, separate the hind limbs of your specimen and *cut* down along the midventral line. The cut will pass through muscle and the cartilaginous pelvic girdle. With your fingers, spread the cut edges apart and use blunt dissecting instruments to separate connective tissue.

Figure 24.9 Fetal pig, female urogenital system.



Ⓢ 2. Three ducts will be seen passing from the body cavity to the posterior surface of the animal. The urethra is ventral to the other 2. The *urethra* leaves the bladder and passes into the *urogenital sinus*. The *rectum* is dorsal to the other 2 ducts. It passes to its own opening, the *anus*.

Ⓢ 3. Find the *vagina,* located dorsally to the urethra. It connects anteriorly to the *uterus* and allows the entrance of sperm in mating and the passage of young pigs at birth. The vagina also enters the *urogenital sinus*. This sinus is absent in adult female pigs and other mammals. As the fetus continues to develop, the urethra and vagina continue to separate posteriorly until finally the *excretory* and *reproductive systems* are completely independent of one another—a distinctive feature of female mammals.

Comparisons

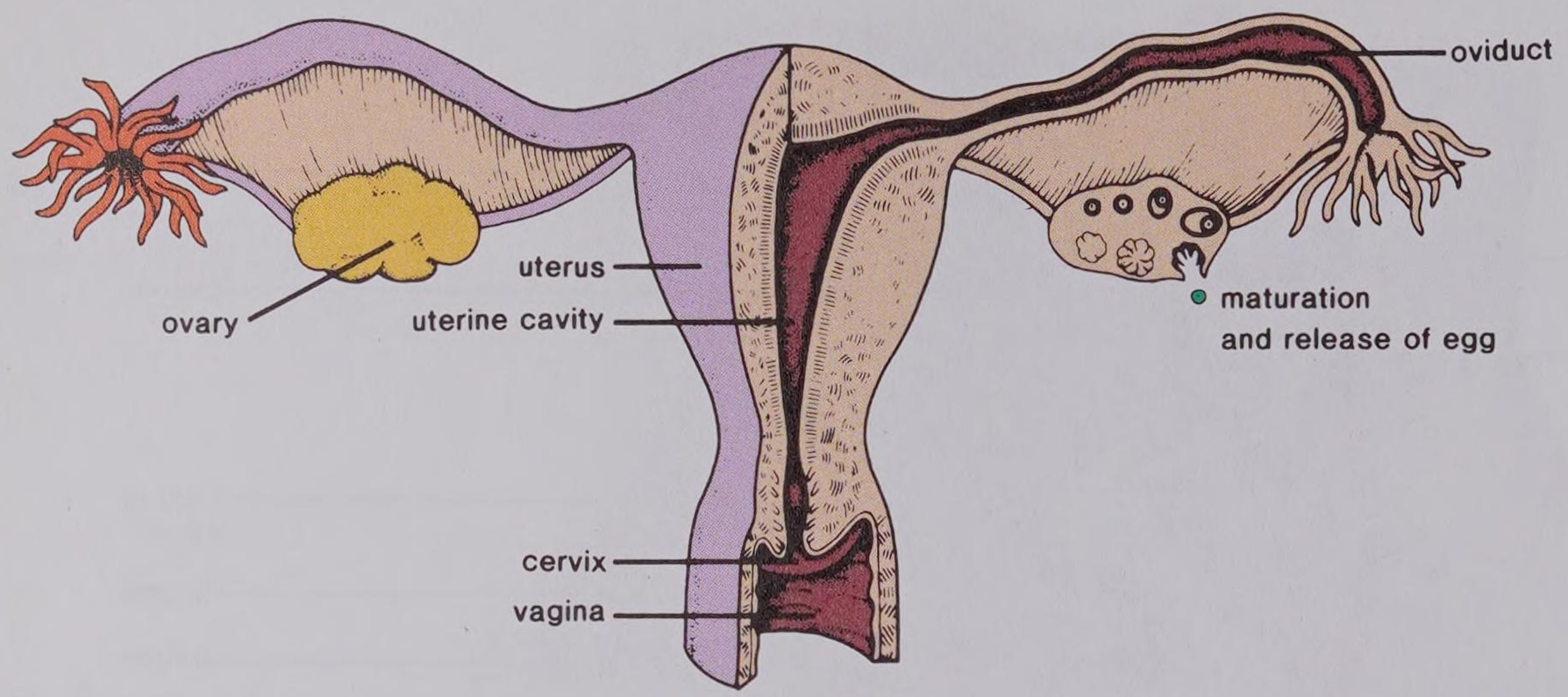
Males Compared to Females

Ⓢ Complete table 24.5 to describe the differences between the male and female reproductive systems.

Table 24.5 Comparison of Male and Female Reproductive Systems

	Males	Females
1. Sex gland		
2. Duct from this gland		
3. Duct passes to what structure?		
4. Final structure in tract		

Figure 24.10 Human female reproductive system.



Fetal Pig Compared to Human

Female Reproduction

Study figure 24.10 and note the fused *uterus* in humans. In table 24.6, compare the human reproductive system to that of the pig.

Table 24.6 Female Reproduction

	Human	Fetal Pig
Oviducts		
Uterus		
Urogenital sinus		

Male Reproduction

Study figure 24.11 and note in table 24.7 the difference in location of the *penis*.

Table 24.7 Male Reproduction

	Human	Fetal Pig
Penis		

Laboratory Review 24

1. Name the organ that follows the one listed:

_____ a. renal artery

_____ b. esophagus

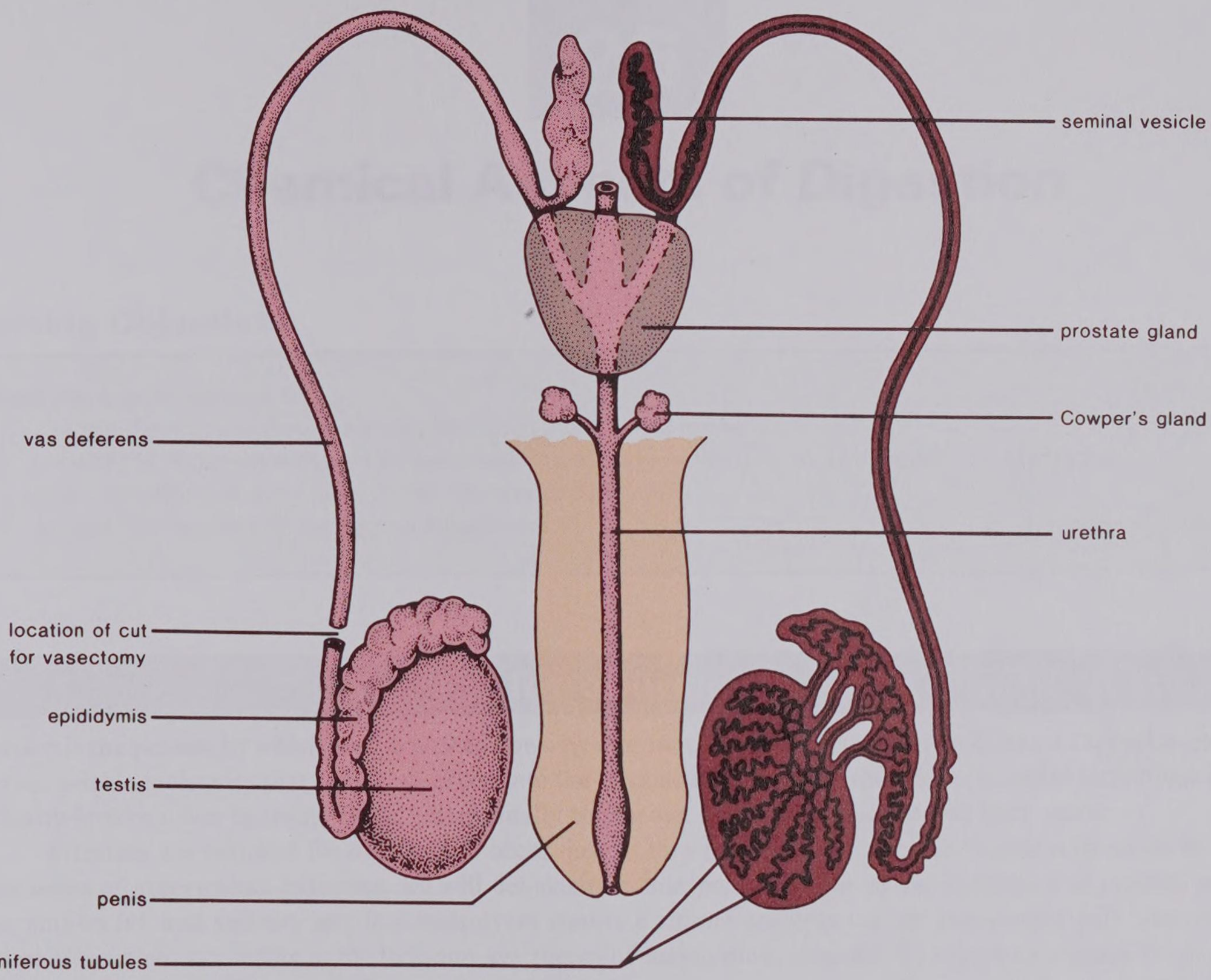
_____ c. ureter

_____ d. vas deferens

_____ e. bronchi

_____ f. pulmonary artery

Figure 24.11 Human male reproductive system.



_____ 2. In birds and mammals, what chambers of the heart contain deoxygenated blood?

_____ 3. Which one animal studied lacks a pulmonary circulatory system?

_____ 4. What is the major difference between the female reproductive systems in pigs and humans?

5. In general, describe the path of blood for the pulmonary and system circuits.

Reference

Brooklyn College of the City University of New York. 1973. *Biology 2.* Dubuque, IA: Kendall/Hunt Publishing Company.

Laboratory

25

Chemical Aspects of Digestion

Learning Objectives

Students should be able to

1. cite the digestive action of salivary amylase, pepsin, and lipase;
2. predict the digestive outcome of combinations of various substances and explain the prediction;
3. state the chemical tests used in this laboratory exercise;
4. explain the function of the control experiment.

Introduction

Digestion is the process by which food is broken down by enzymatic hydrolysis (a reaction of water that splits compounds apart) to soluble molecules that can be absorbed into the bloodstream. For example, water is added to proteins until they are finally broken down to amino acids, starch finally to glucose, and fats to glycerol and fatty acids.

Enzymes are required for digestion to occur, just as they are required for other chemical reactions in the body. In the series of experiments following, we will demonstrate that *pepsin* speeds up the hydrolysis of protein, *pancreatic lipase* acts on fat, and *salivary amylase* hydrolyzes starch. Enzymes are very specific and usually will take part in only one type of reaction. According to the lock-and-key theory of enzymatic action, this is because an enzyme fits its substrate just as a key fits a lock. It is important to realize that enzymes must maintain an appropriate shape or configuration to take part in a reaction. Enzymes have a preferred pH because this pH maintains their usual 3-dimensional shape. Enzymatic reactions speed up with increased temperature but are destroyed by excessive heat or boiling.

A well-designed experiment attempts to hold all experimental conditions constant except the one being tested (*experimental variable*). A *control* is an experiment in which all steps of the reaction (experiment) are included *except* the experimental variable. If the experiment in fact has only one variable, and all of the chemical (materials) are performing as expected, the results of this *control* should be negative. Often a second control is performed in which a material that is known to be positive is tested to check that the chemicals (tests) are functioning as they should; these are *positive controls*. In experiments such as the ones you are conducting today, many variables affect their outcome. Some of these are purity of the reagents (chemical); age of the reagent, particularly organic compounds, which can deteriorate upon storage; conditions of storage—was the reagent stored in the correct environment, that is, dry, refrigerated, frozen; if the experimental variable is a living organism, is it healthy, about to enter dormancy, wilted, young, or actively growing?

Protein Digestion by Pepsin

Digestion of protein by pepsin takes place in the stomach and may be described by this reaction:

$$\text{protein} + \text{water} \xrightarrow{\text{pepsin (enzyme)}} \text{n peptides}$$

Note the placement of a material on *top* of the reaction arrow indicates that the material is not consumed or changed in the process. Enzymes are recycled.

The stomach has a very low pH. This would indicate that pepsin works effectively in which environment (acid or base)? _____

In the following experiment, *biuret reagent* is used to test for the presence of protein and peptides. In the presence of protein, biuret reagent turns *violet;* in the presence of polypeptides, biuret reagent turns *pinkish.* To do the test, simply add an equal number of drops of biuret reagent to each tube.

Experiment

Label and mark 4 test tubes at the 2 cm, 4 cm, and 6 cm levels and fill as follows.

- ❶ ❸ Tube 1: Fill to the 4 cm mark with *water* and to the 6 cm mark with *1%–2% pepsin* solution. Shake. Incubate at 37° C for 2 hours.
- ❶ ❸ Tube 2: Fill to the 2 m mark with *albumin* solution, to the 4 cm mark with *pepsin* solution, and to the 6 cm mark with *water.* Shake. Incubate at 37° C for 2 hours.
- ❶ ❸ Tube 3: Fill to the 2 cm mark with *albumin* solution, to the 4 cm mark with *pepsin* solution, and to the 6 cm mark with *0.2% HCl.* Shake. Incubate at 37° C for 2 hours.
- ❶ ❸ Tube 4: Fill to the 2 cm mark with *albumin* solution, to the 4 cm mark with *pepsin* solution, and to the 6 cm mark with *0.2% HCl.* Shake. Keep at room temperature (≈ 22° C).
- ❸ After 2 hours, add an equal number of drops of *biuret reagent* to each tube. Record and explain your results. (table 25.1).

Table 25.1 Protein Digestion

Tube No.	Contents	Temperature (° C)	Results of Test	Explanation
1				
2				
3				
4				

Fat Digestion by Pancreatic Lipase

Digestion of oil takes place in the small intestine and may be described by these 2 reactions:

$$\text{(1) fat} \xrightarrow[\text{bile (emulsifier)}]{} \text{fat droplets}$$

$$\text{(2) fat droplets} + \text{water} \xrightarrow[\text{lipase (enzyme)}]{} \text{glycerol} + 3 \text{ fatty acids}$$

The first reaction is not an enzymatic reaction; it is an emulsification reaction in which fat is physically dispersed into small droplets. Bile is the emulsifier that causes this to happen. Explain the need for bile before fat digestion takes place.

Considering the second reaction, would the pH of the solution be lower before or after the reaction? _____

The test for fat digestion in the experiment following is a change in a pH indicator as the solution in the test tube goes acid. Phenol red turns from *red* to *yellow* as the conditions become acid.

Experiment

- ❸ 1. Label and mark 3 test tubes at the 3 cm and 6 cm levels.
- ❸ 2. Add *1%–2% pancreatin* (containing pancreatic lipase) to each tube to the 3 cm mark.
- ❸ 3. Complete each tube as follows.

S Tube 1: Fill to the second mark with *bile* solution.

S 1. Add 2 or 3 drops of *phenol red* and then drops of *0.2% NaOH* until the tube turns pink.
S 2. Add 5 drops of *oil*.
I S 3. Incubate at 37° C for 1 hour.
S 4. *Shake* initially and occasionally thereafter.
S 5. Observe and *record* the initial color and final color in table 25.2.

S Tube 2: Fill to the second mark with *water*.

S 1. Add the same number of drops of *phenol red* as previously and then drops of *0.2% NaOH* drop by drop until the tube just starts to turn pink.
S 2. Add 5 drops of *oil*.
I S 3. Incubate at 37° C for 1 hour.
S 4. *Shake* initially and occasionally thereafter.
S 5. Observe and *record* the initial color and final color in table 25.2.

S Tube 3: Fill to the second mark with *bile* solution.

S 1. Add the same number of drops of *phenol red* as earlier and then drops of *0.2% NaOH* drop by drop until the tube just starts to turn pink.
S 2. Add 6 drops of *oil*.
I S 3. Allow the tube to remain at room temperature ($\approx$ 22° C) for 1 hour.
S 4. *Shake* initially and occasionally thereafter.
S 5. Observe and *record* the initial color and final color in table 25.2.

S Tube 4: Construct your own *control* for this experiment.

S 1. Enter the contents of your control tube into table 25.2.
S 2. Observe and record the initial color and final color in table 25.2.

Table 25.2 Fat Digestion

Tube No.	Contents	Temperature (° C)	Color		Explanation
			Initial	Final	
1					
2					
3					
4					

Starch Digestion by Amylase

Digestion of starch by salivary amylase takes place in the mouth and may be described by this reaction:

$$\text{starch} + \text{water} \xrightarrow{\text{amylase (enzyme)}} \text{n maltose}$$

Why is this, like the other reactions studied today, called a hydrolytic reaction? _____

_____

If digestion does *not* occur, which will be present—starch or maltose? _____

If digestion does occur, which will be present—starch or maltose? _____

General Experimental Directions

In the experiment that follows, 2 tests for starch digestion are used. If no digestion has taken place, the *iodine* test for starch will be positive. This test for starch is simply to add a few drops of *iodine* to the tube. If starch is present, a blue-black color immediately appears.

If digestion has taken place, a test for maltose (sugar) will be positive. To test for sugar (maltose), add an equal amount of *Benedict's* solution to your test tube. Place it in a *boiling water bath* for 5 minutes and note any color change. A color change from blue → green → yellow → orange → red indicates the presence of maltose.

The final 5-minute boiling of the test tubes with *Benedict's* solution is necessary for it to react; it should not influence the results.

To which category of organic compound (lipid, carbohydrate, protein) do enzymes, such as amylase, belong?

_____

Recalling Laboratory 2 or from reading your text, what happens to these kinds of chemicals when they are drastically treated (boiled, acid treated, etc.)? _____

Experiment

Ⓢ 1. Label and mark 6 test tubes at 1 cm and 2 cm. Fill tubes 1–5 to the first mark with *alpha-amylase* solution. Fill tube 6 to the first mark with *water*.

Ⓢ 2. *Boil* the *alpha-amylase* solution in test tube 5 by placing the tube in a *boiling water bath* for 10 minutes. While this is boiling, complete the work for test tubes 1 and 2 as follows.

Ⓢ Tube 1: Fill to the second mark with *starch* solution and *test for starch* (iodine) *immediately*. Record and explain your results in table 25.3.

Ⓢ Tube 2: Fill to the second mark with *starch* solution and *test for sugar* (Benedict's plus boiling) *immediately*. Record and explain your results in table 25.3.

Ⓢ 3. Complete the work for test tubes 3–6 as follows.

Ⓘ Ⓢ Tube 3: Fill to the second mark with *starch* solution. Place in a test tube rack on your desk, and allow it to react (22° C ≈ room temperature) for 30 minutes. Test for *starch* (iodine). Record and explain your results in table 25.3.

Ⓘ Ⓢ Tube 4: Fill to the second mark with *starch* solution. Place in a test tube rack on your desk, and allow it to react (22° C ≈ room temperature) for 30 minutes. Test for *sugar* (Benedict's plus boiling). Record and explain your results in table 25.3.

Ⓘ Ⓢ Tube 5: Fill to the second mark with *starch* solution. Place in a test tube rack on your desk, and allow it to react (22° C ≈ room temperature) for 30 minutes. *Divide* the contents of the tube in half. Test (a) for *sugar* (Benedict's plus boiling) and (b) for *starch* (iodine). Record and explain your results in the table 25.3.

Ⓘ Ⓢ Tube 6: Fill to the second mark with *starch* solution. Place in a test tube rack on your desk, and allow it to react (22° C ≈ room temperature) for 30 minutes. Test for sugar (Benedict's). Record and explain your results in table 25.3.

Table 25.3 Starch Digestion

Tube No.	Contents	Test Done	Results	Explanation
1				
2				
3				
4				
5a				
5b				
6				

Which test tube(s) serves as a control(s) in this experiment? _____

Summary

Explain how each of these requirements influences effective digestion (table 25.4).

Table 25.4 Requirements for Digestion

Requirement	Explanation
Specific enzyme	
Specific substrate	
Warm temperature	
Time	
Specific pH	
Fat emulsifier	

Control

A control goes through all the steps of a reaction except the one being tested (i.e., a control sample is not exposed to the experimental variable). What is the function of a control? _____

_____

Laboratory Review 25

_____ 1. Why does salivary amylase function better in the mouth than in the stomach?

_____ 2. Is the pH requirement for the digestion of protein in the stomach low, neutral, or high?

_____ 3. Why does phenol red turn from red to yellow as oil is digested?

_____ 4. What is the optimum temperature, in degrees Celsius (centigrade), for the action of enzymes in humans?

_____ 5. Benedict's test is used to detect the presence of what molecule? Describe this test.

6. Why did you perform 2 tests to determine that starch had been digested?

7. Describe the recommended test used to detect the presence of proteins.

8. What is a control? Why do you need it when you are performing an experiment?

Laboratory

26

Homeostasis

Learning Objectives

Students should be able to

1. describe the role played by the lungs, liver, and kidneys in homeostasis;
2. state the functions of the lungs, liver, and kidneys studied in the laboratory exercise;
3. describe the microscopic anatomy of the lung and kidney;
4. trace the path of blood to and away from the liver, to and away from a kidney, and about a kidney tubule;
5. predict whether substances will be in the filtrate and/or urine and explain;
6. name the parts of a kidney tubule and how each part relates to the 3 steps in urine formation.

Introduction

Homeostasis refers to the constancy of the internal environment. The internal environment of vertebrates, including humans, is the blood and tissue fluid. Tissue fluid is continually created and refreshed at the capillaries when certain molecules leave blood at the arterial side and others are picked up by blood at the venous side of a capillary, as illustrated in figure 26.1.

What type of pressure continually causes water to exit from the arterial side of the capillary? _____

Other molecules diffuse out of the capillary at the arterial side. What are these molecules? _____

What type of molecule diffuses into the capillary at the venous side? _____

What type of pressure continually causes water to enter the venous side of the capillary? _____

Figure 26.1 Diagram of a capillary, illustrating the exchanges that take place and the forces that aid the process.

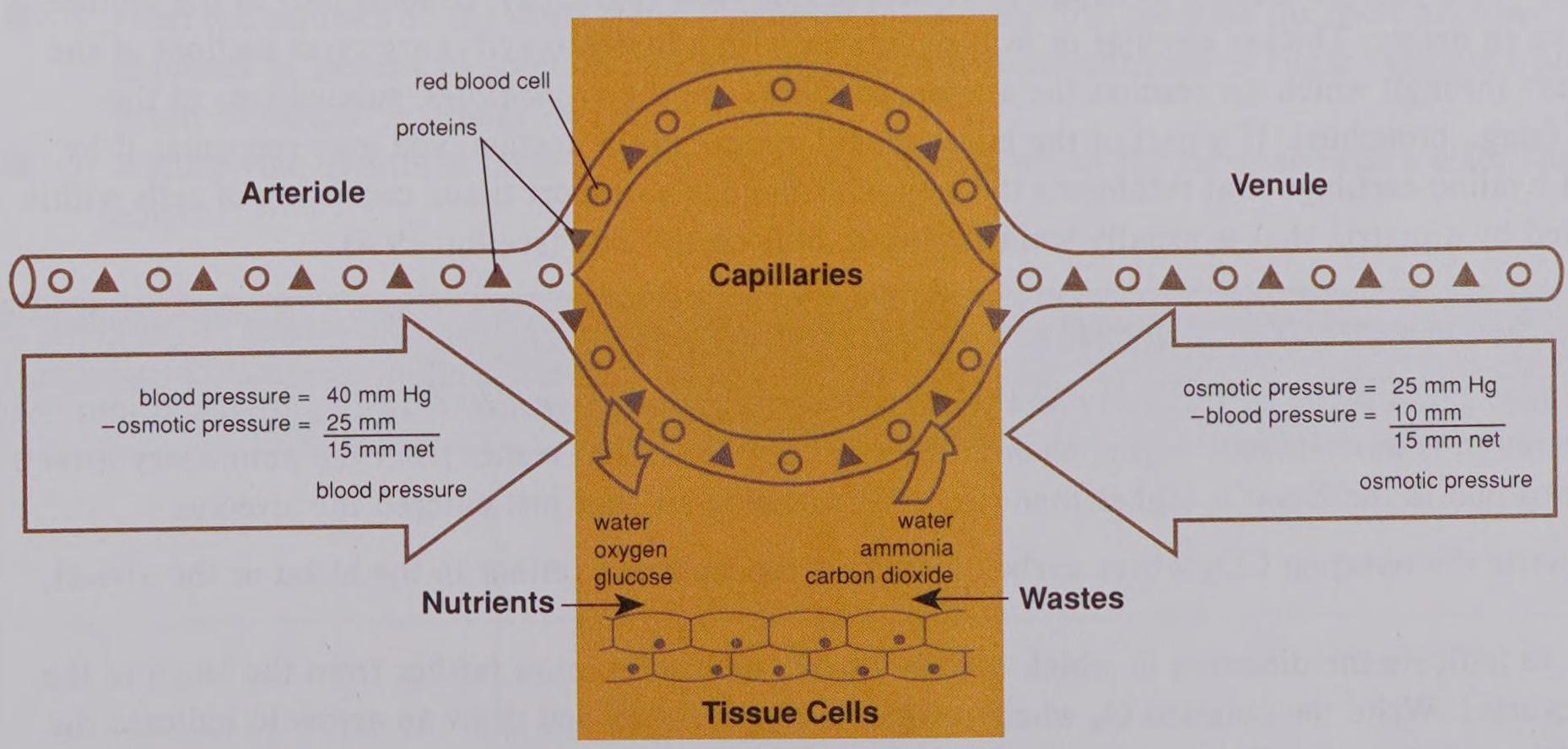
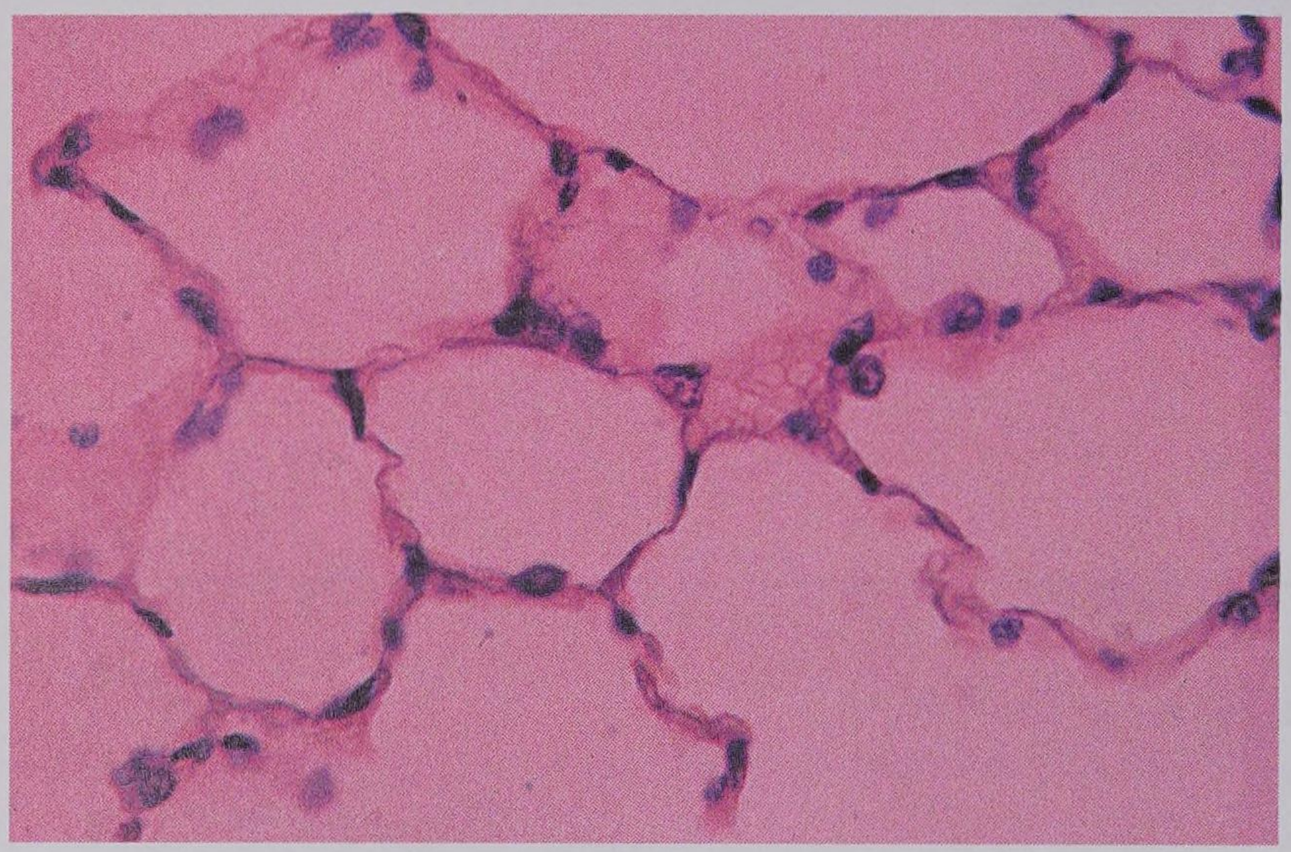


Figure 26.2 Alveoli of lung.



Organs of Homeostasis

All internal organs contribute to homeostasis. In this laboratory, we study the *lungs,* the *liver,* and the *kidneys* to determine the role they play in supplying the blood (and cells) with glucose and oxygen and in removing carbon dioxide and nitrogenous wastes from the blood.

Lungs

Microscopic Structure of the Lung

The vertebrate lung is a spongy organ consisting of irregularly shaped air spaces called *alveoli* (sing., alveolus). The alveoli are lined by a single layer of flat cells and supported by a mesh of fine elastic fibers. The alveoli are surrounded by a rich network of *pulmonary capillaries.*

S 1. Obtain a slide with a stained section of a *lung.* In stained slides, the nuclei of the cells forming the thin alveolar walls appear purple or dark blue.

S 2. Look for the areas where there are groups of red or orange disc-shaped *erythrocytes* (red blood cells). When they appear in strings, you are looking at capillary vessels in side view (fig. 26.2). In some part of the section, you may observe an *artery.* Thicker circular or oval structures with a lumen (cavity) are cross sections of the tubular pathways through which air reaches the air spaces. These are the *bronchioles,* subdivisions of the larger *bronchi* (sing., bronchus). If a part of the latter should appear in the section, you may recognize it by the presence of hyaline cartilage that reinforces the wall. Cartilage is a support tissue consisting of cells within spaces, separated by a matrix that is usually stained pink or blue on the side (see fig. 29.4).

Gas Exchange between the Capillary and the Alveolus

Gas exchange in the lungs takes place by *diffusion* and follows the *concentration gradient.* In the air taken into the alveoli, oxygen concentration is momentarily higher than in the capillary blood that comes from the pulmonary artery. Carbon dioxide concentration in the blood is higher than that of the outside air that just entered the alveolus.

S 1. In figure 26.2, *write* the notation CO_2 where carbon dioxide is concentrated (either in the blood or the alveoli, as appropriate.

S 2. Draw an *arrow* to indicate the direction in which carbon dioxide molecules move (either from the blood to the alveoli or the reverse). Write the notation O_2 where oxygen is concentrated and draw an arrow to indicate the direction in which oxygen molecules move.

S 3. State here one way in which the lungs contribute to homeostasis. _____

_____

Carbon dioxide is carried in the blood as the bicarbonate ion HCO_3^-:

$$CO_2 + H_2O \rightarrow H_2CO_3 \rightarrow HCO_3^- + H^+$$

Is blood more acid when it is carrying carbon dioxide? _____

How do you know? _____

When carbon dioxide leaves the blood, the bicarbonate ion breaks down to carbon dioxide and water:

$$HCO_3^- + H^+ \rightarrow H_2CO_3 \rightarrow H_2O + CO_2$$

Is blood less acid when the carbon dioxide exits? _____

S 4. State here another way in which the lungs contribute to homeostasis. _____

_____

Liver

Blood Flow

The products of digestion—amino acids and glucose—are absorbed by the *villi* (small, fingerlike projections) lining the wall of the small intestine. Thereafter, these products enter the blood capillaries and are transported by the *hepatic portal vein* to the liver. Because of this, the liver can regulate the entrance of molecules into the main bloodstream.

S With the aid of figure 26.3, *trace* the *path* of blood from the heart to the intestine, then to the liver, and finally back to the heart. Fill in the following sequence:

1. Left ventricle
2. _____
3. _____
4. Intestine
5. _____
6. _____
7. _____
8. _____
9. Right atrium

Role of Liver in Maintaining Glucose Concentration of Blood

S 1. Test the glucose concentration of 3 samples of (artificial) serum (as if) taken from an animal *soon after eating*. The samples are taken from these 3 locations: A. *mesenteric artery,* B. *hepatic portal vein,* C. *hepatic vein.*

S 2. Label and mark off 3 test tubes at 2 cm and 4 cm.

S 3. Fill to the first mark with *experimental serum* A_1 B_1, or C_1 and to the second mark with *Benedict's* solution.

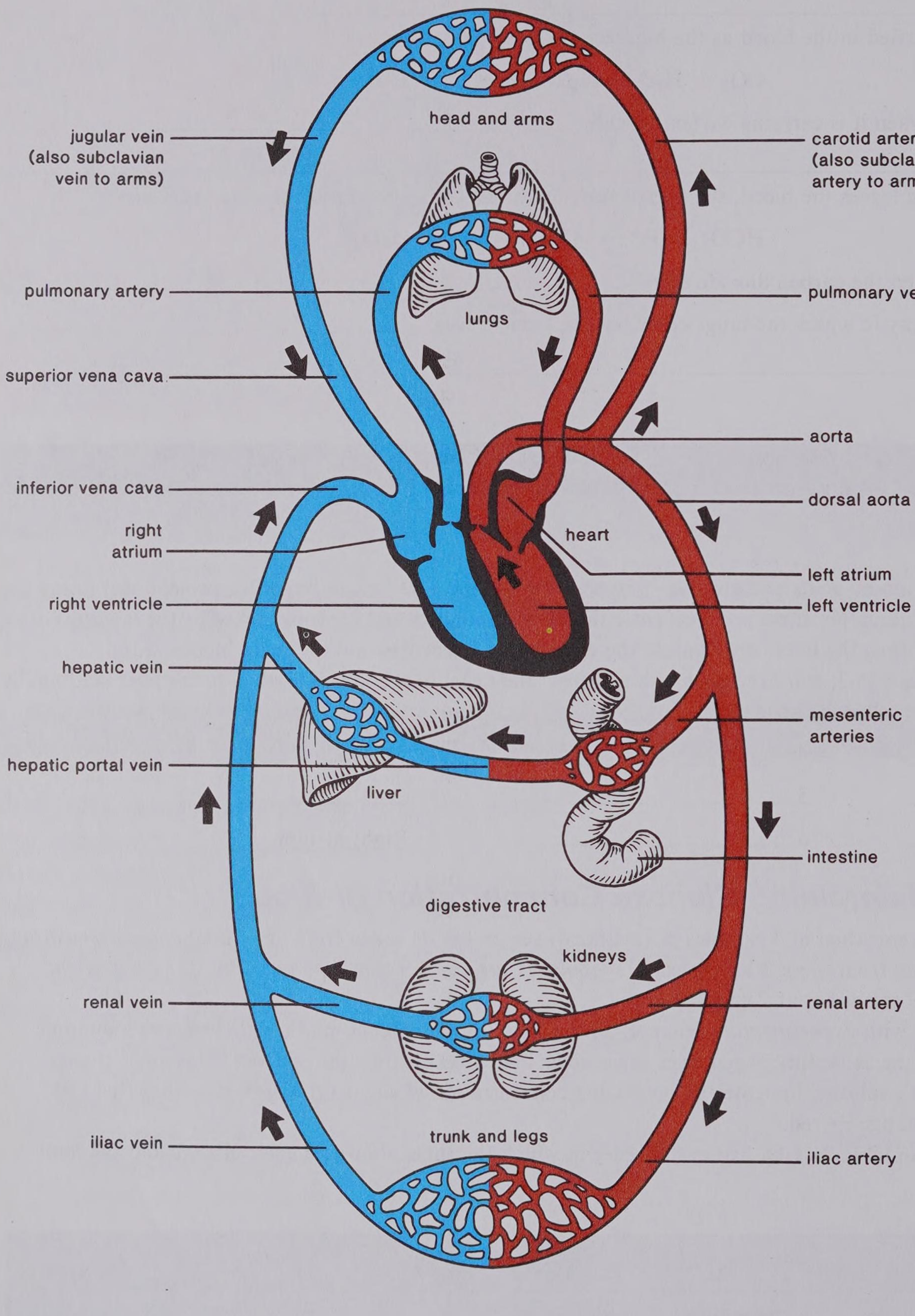
S 4. Heat the samples at the same time; whichever turns color first contains the most glucose. This color change sequence of Benedict's solution indicates an increasing concentration of sugar (glucose): blue (negative) → green → yellow → orange → red.

S 5. Record your results in table 26.1 by listing the order in which the tubes showed a color change and the final color.

Table 26.1 Glucose Concentration in Blood (soon after eating)

Order of Tubes	Final Color	Serum from Where?

Figure 26.3 Diagram showing the major blood vessels in the body and the flow of blood.



Which blood vessel, mesenteric, hepatic portal vein, or hepatic vein, contains the most glucose soon after eating?

_____

Why do you suppose the hepatic vein does not contain as much glucose as the hepatic portal vein soon after eating?

_____

_____

1. To complete your study, test the glucose concentration of 3 samples of (artificial) serum (as if) taken from the same 3 blood vessels of an animal that has *not eaten* for 6–8 hours.
2. Label and mark off 3 test tubes at 2 cm and 4 cm.
3. Fill to the first mark with *experimental serum* A_2, B_2, C_2 and to the second mark with *Benedict's* solution. Heat the samples at the same time; the sample that turns color first contains the most glucose.
4. Record your results in table 26.2 by listing the order in which the tubes showed a color change and the final color.

Table 26.2 Glucose Concentration in Blood (not eaten in 6–8 hours)

Order of Tubes	Final Color	Serum from Where?

Which blood vessel, mesenteric, hepatic portal vein, or hepatic vein contains the most glucose between eating?

_____

Why do you suppose the hepatic vein now contains more glucose than does the hepatic portal vein? _____

_____

Storage of Glucose as Glycogen

Just after eating, the liver stores excess glucose as glycogen. In between eating, (6–8 hours after having eaten), glycogen is broken down by liver cells to produce glucose, and this glucose enters the bloodstream to maintain the blood sugar level at 0.1%.

1. In the following equation, write the words *after eating* and *between eating* on the appropriate line:

_____ eating

n glucose $\rightleftharpoons$ glycogen

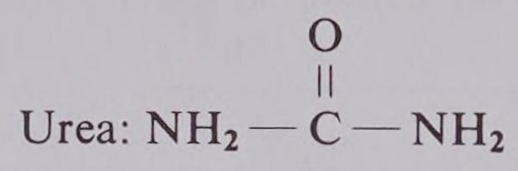
_____ eating

2. The hormone *insulin,* made by the pancreas, promotes the uptake and storage of glucose in the liver. Write the word *insulin* by the appropriate arrow. If the pancreas should cease making insulin, glucose is excreted in the urine instead of being stored. The individual then has the medical condition called *diabetes mellitis.*
3. State one way in which the liver contributes to homeostasis. _____

_____

Urea

The liver removes ammonia (NH_3) from the blood and converts it to urea, a substance less toxic to humans. The chemical formula for urea follows. Circle that portion that would have come from ammonia (NH_3).



Urea: $NH_2 — C(=O) — NH_2$

State another way in which the liver contributes to homeostasis. _____

_____

Kidneys

Macroscopic Anatomy

The human kidneys are bean-shaped organs that lie along the dorsal wall of the abdominal cavity.

Study the model of the kidney and locate the outer covering or *cortex,* the inner *medulla,* and the *pelvis,* where urine collects (fig. 26.4).

Microscopic Anatomy

Each human kidney contains about one million *nephrons* (kidney tubules). With the help of figure 26.5, list the portions of a kidney tubule. _____

_____

Which of these portions are in the cortex? _____

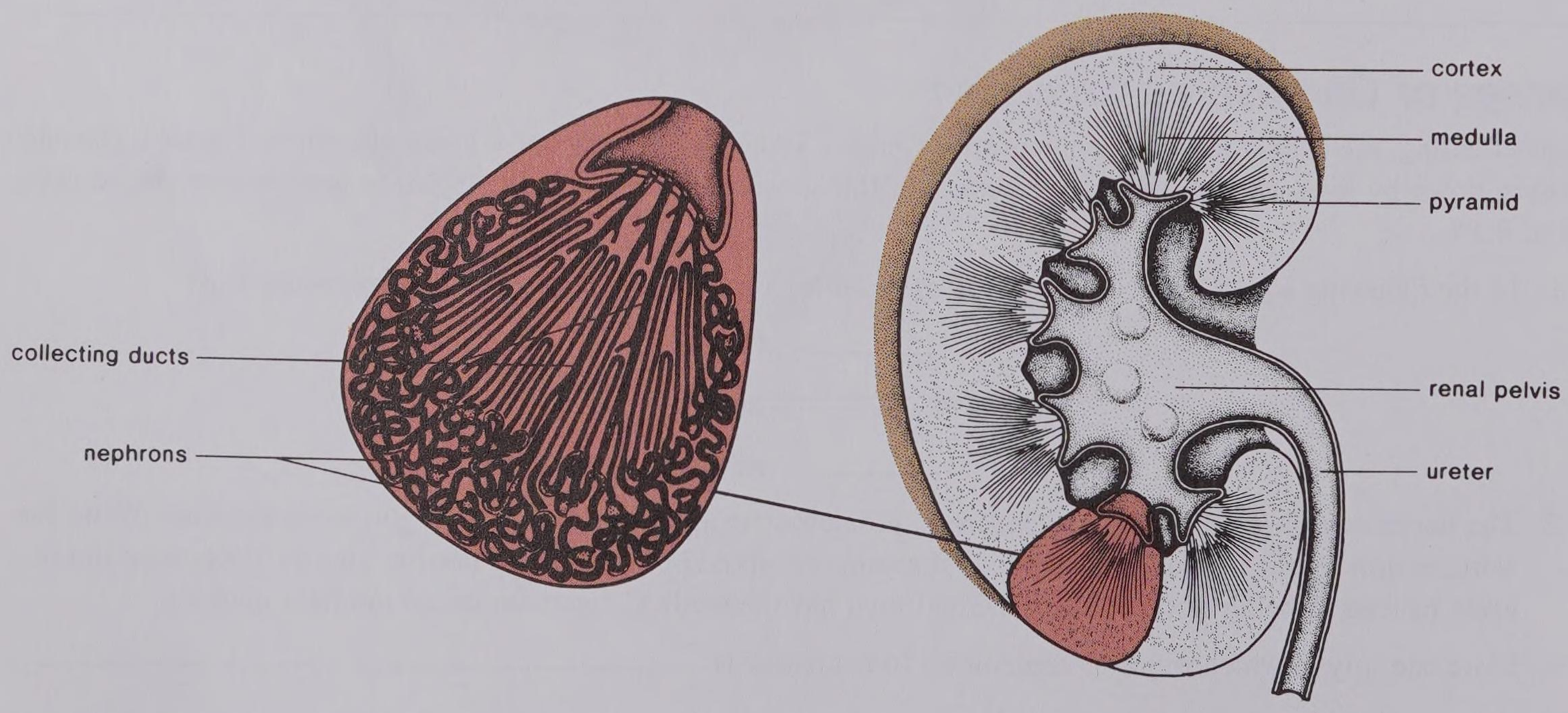
Which are in the medulla? _____

With the help of figure 26.5, trace the path of blood toward, around, and away from an individual tubule. ____

_____

_____

Figure 26.4 Kidney, longitudinal section, with a blowup of a pyramid.



Urine Formation

Urine formation requires 3 steps: filtration, reabsorption, and tubular excretion. *Filtration* requires the movement of molecules from the glomerulus to the inside of the glomerular capsule.

Ⓢ Mark the location of filtration (FIL) in figure 26.5.

Reabsorption requires the movement of molecules from the proximal convoluted tubule to the peritubular capillary network.

Ⓢ Mark the location of reabsorption (RE) in figure 26.5.

Tubular excretion requires the movement of molecules from the peritubular capillary network to the distal convoluted tubule.

Ⓢ ⒶMark the location of tubular excretion (Tub. ex.) in figure 26.5.

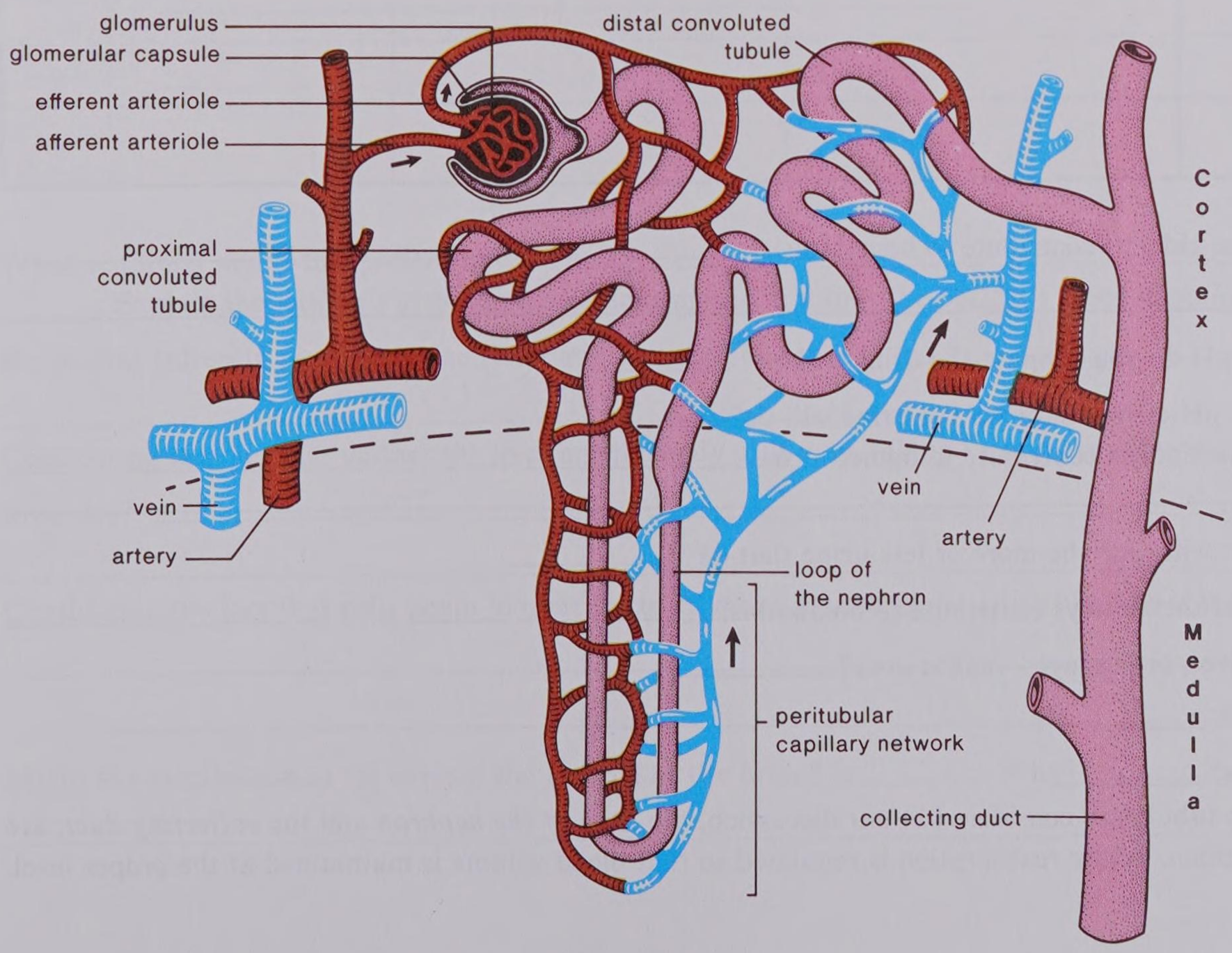
Filtration

When blood enters the glomerulus, it contains the following substances listed. Blood pressure causes small molecules to exit from the blood and enter the glomerular capsule. Water, amino acids, salts, urea, and water are small molecules.

Ⓢ Draw an arrow from the left to the right for all molecules that enter the glomerular capsule and are called the *filtrate*.

Glomerulus	**Glomerular Capsule**
Cells	
Proteins	
Glucose	
Amino acids	
Salts	
Urea	
Water	

Figure 26.5 Kidney tubule.



Reabsorption

When the filtrate enters the proximal convoluted tubule, it contains the following substances listed. The cells that line the proximal convoluted tubule are engaged in active transport and have the capability to reabsorb nutrients and whatever water and salts are needed to maintain the blood volume and pH. ❶ Draw an *arrow* from the left to the right for all molecules that *maximally reenter* the blood.

Proximal Convoluted Tubule	**Peritubular Capillary**
Water	
Glucose	
Amino acids	
Urea	
Salts	

Tubular Excretion

Some substances, like penicillin and certain acids and bases, are actively excreted from the peritubular capillary into the fluid of the distal convoluted tubule. This is a way for the body to rid itself of a waste that was not filterable at the glomerular capsule.

Summary

Consider the substances listed in table 26.3. *Check* off those that you expect to be in *blood,* which in the *filtrate,* and which in *urine?*

Table 26.3 Urine Formation

	In Blood	**In Filtrate**	**In Urine**
Protein (albumin)			
Glucose			
Urea			
Water			

State one way in which the kidneys contribute to homeostasis. _____

_____

If the blood is acid, what pH do you suppose the urine will be? _____

If the blood is basic, what pH do you suppose the urine will be? _____

State here another way the kidneys contribute to homeostasis. _____

_____

If you drink a lot of water, will there be more or less urine that day? _____

State still another way that the kidneys contribute to homeostasis. _____

What organ—the lungs, liver, or kidneys—makes urea? _____

What organ excretes urea? _____

What organ excretes urine? _____

The 2 parts of the tubule *not* considered in our discussion, the *loop of the nephron* and the *collecting duct,* are both active in water absorption. Water reabsorption is regulated so that blood volume is maintained at the proper level.

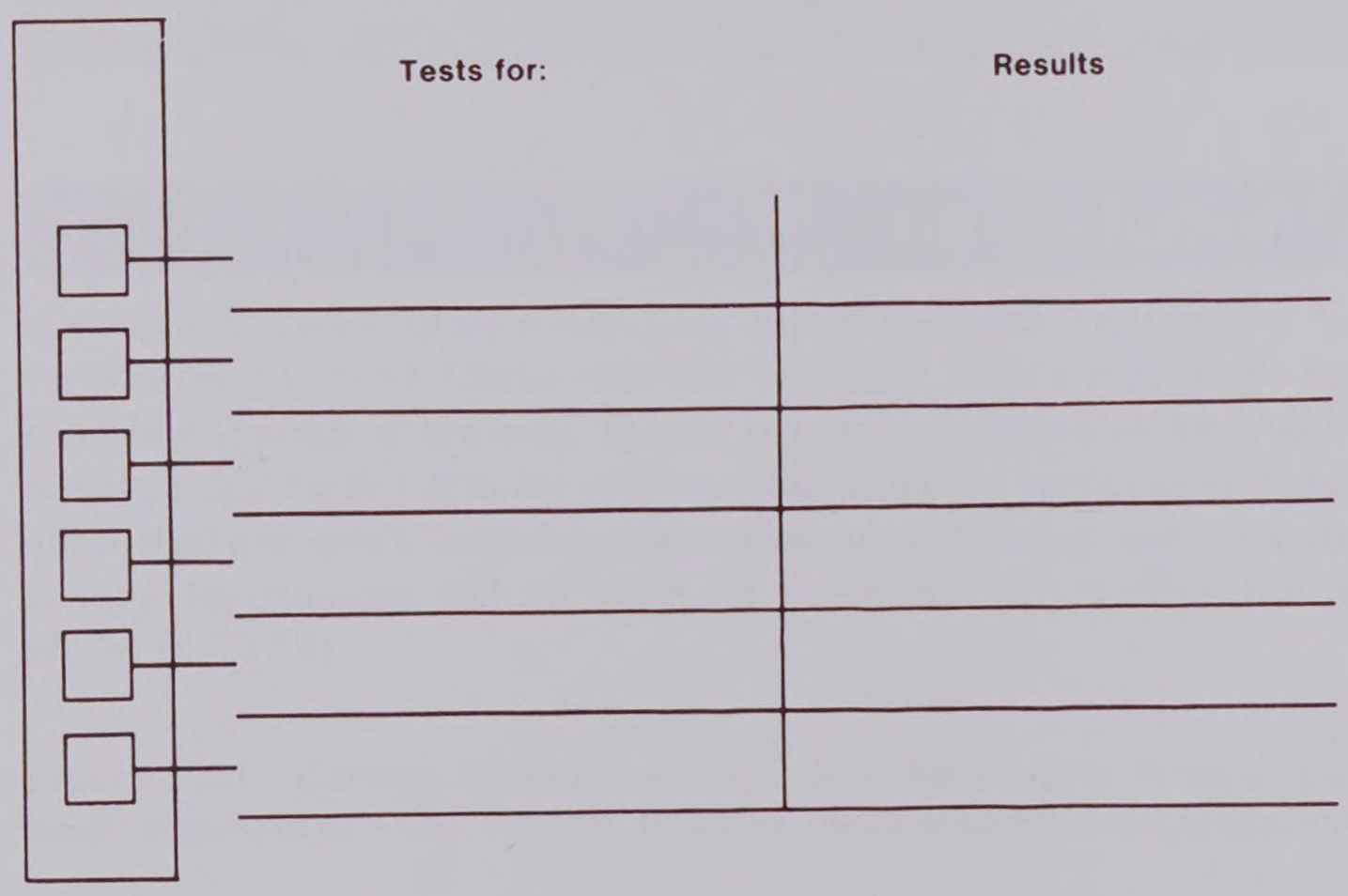
Urinalysis

Urinalysis can help determine the illness of a patient. Suppose a patient complains of excessive thirst and urination; loss of weight despite an intake of sweets; feeling tired and run down. A urinalysis has been ordered, and you are to test the urine.

A urinalysis is easily done by using a dipstick, which is a plastic strip containing reagent squares that produce specific color reactions in the presence of certain substances in the urine.

1. Obtain a *dipstick* and determine the location of the specific reactions on the strip.
2. Examine the *chart* on the container, which indicates the respective color changes.
3. Write beside the drawing in figure 26.6 the tests for which the dipstick may be employed.
4. Mark a test tube at 10 cm and fill to the mark with the "patient's (artificial) urine." Completely, but briefly, immerse all reagent areas of the dipstick in the urine.
5. Drain the dipstick on a paper towel to remove excess solution. Read your results according to the chart on the container and record your results on the lines provided (fig. 26.6).

Figure 26.6 Dipstick.



What condition might this patient have? _____

Explain the patient's symptoms. Considering the fact that the patient's blood contains excess glucose, why was the patient suffering from excessive thirst and urination? _____

_____

Considering the fact that neither the liver nor the body cells were taking up glucose, why was the patient suffering from tiredness? _____

_____

Considering the fact that cells begin to metabolize fat when glucose is not available, why was this patient losing weight?

_____

_____

Might the metabolism of fat explain the low pH of the urine? _____ Why? _____

_____

Laboratory Review 26

_____ 1. What process accounts for the exchange of gases in the lungs?

_____ Does this process require energy?

_____ 2. What molecule is excreted by the lungs?

_____ 3. How is carbon dioxide carried in the blood from the cells to the lungs?

_____ 4. What organ stores glucose and then releases it into the bloodstream?

_____ 5. How is glucose stored in the bodies of animals?

_____ What hormone is responsible for the uptake and storage of glucose?

_____ 6. What condition develops if an individual lacks the hormone given as an answer in question 5?

7. What are the 3 processes required for urine formation, and what substances of interest are involved in each process?

Reference

Schmude, J. 1981. Clinical urinalysis: Implications for teaching. *American Biology Teacher* 43(3):165.

Laboratory

27

The Nervous System

Learning Objectives

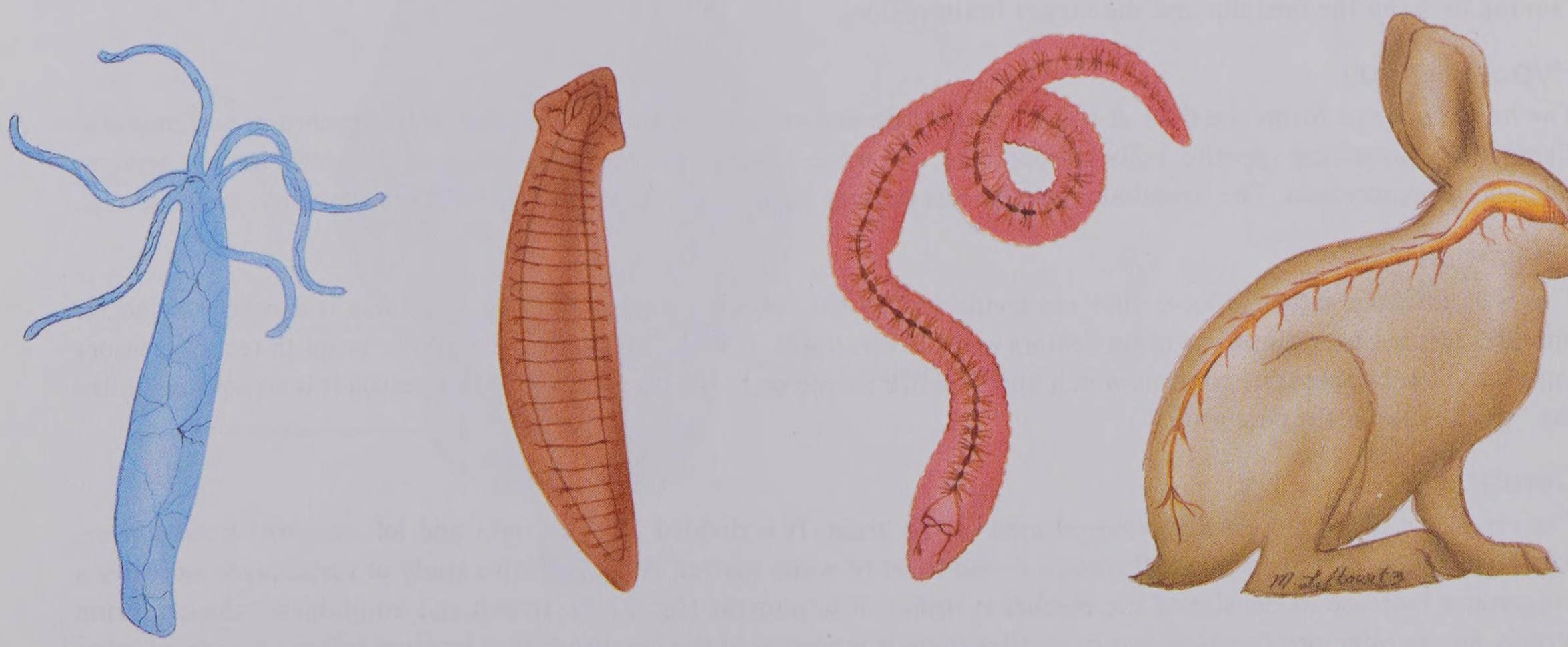
Students should be able to

1. identify the parts of the brain mentioned and state their function;
2. describe the anatomy of the spinal cord;
3. describe the anatomy and physiology of a spinal reflex arc;
4. predict the effects of damage to regions of the arc;
5. identify the parts of the eye, ear, and skin and state a function for each part.

Introduction

A comparative study of animal nervous systems indicates the steps that may have led to the complex nervous system of vertebrates (fig. 27.1). Cnidarians, such as *Hydra,* have a very simple nervous system that looks like a net of threads extending throughout the body. The *net* is actually composed of *neurons* (nerve cells), each of which has *processes* (extensions) that reach out to the other neurons. Impulses spread in all directions along the net, which seems primarily to allow *Hydra* to give a local response to a stimulus. External *receptor cells* that respond to stimuli such as chemicals or pressure communicate with the nerve net, which then causes cells to contract within the 2 layers making up the body of *Hydra* (fig. 17.4).

Figure 27.1 Comparative nervous systems. ***a.*** Nerve net of *Hydra*. ***b.*** Ladder-type organ of a planarian. ***c.*** Ventral solid nerve cord of an earthworm. ***d.*** Dorsal nerve cord and enlarged brain of a rabbit.



a. Hydra b. Planarian c. Earthworm d. Rabbit

Planarians have a more complicated nervous system. They have a head with sense organs and a brain, a concentration of neurons that apparently acts as a relay station between sense organs and muscles. Two longitudinal *nerve cords* with cross-links between them connect the brain to muscles and other parts of the body.

Segmented worms, such as earthworms, have an even more elaborate nervous system. The brain and a large *ventral nerve cord* running along the midline of the body form a central nervous system that is responsible for integration and coordination. The nerve cord gives off nerves in each segment. These nerves constitute a so-called *peripheral nervous system.*

When *invertebrate* nervous systems are compared to vertebrate systems, it is apparent that further evolutionary changes involved vast increases in the number of neurons. For example, an insect's entire nervous system may contain a total of about one million neurons, while a vertebrate nervous system may contain many thousand to several billion times that number.

Brain

The *brain* is a part of the central nervous system. Where is the central nervous system located in an animal's body (fig. 27.1)? _____

Mammalian Brain

The mammalian brain is the enlarged anterior end of the spinal cord (fig. 27.2). The detailed anatomy of the brain is exceedingly complex. We shall consider only 6 main regions—*medulla oblongata, cerebellum, pons, hypothalamus, thalamus,* and *cerebrum*—plus the *ventricles.*

Medulla Oblongata

The *medulla oblongata* is the most posterior portion of the brain stem. Neuron processes pass from the spinal cord through the medulla to higher brain regions. This portion of the brain controls internal organs; for example, cardiac and breathing control centers are present in the medulla.

Cerebellum

The *cerebellum* is located just posterior to the cerebrum, as you observe the brain dorsally. Its 2 lobes give it an appearance rather like a butterfly. In cross section, it has an internal pattern that looks like a tree. The cerebellum functions below the level of consciousness in coordinating equilibrium and motor activity.

Pons

The *pons* is recognized by its ventral, bulblike enlargement on the brain stem. It serves as a passageway for neural fibers running between the medulla and the higher brain regions.

Hypothalamus

The *hypothalamus* forms the floor of the third ventricle and communicates with the thalamus, cerebrum, and medulla. Centers for controlling appetite, body temperature, and water balance are present in the hypothalamus, and its primary function is homeostasis. The hypothalamus also has centers for pleasure, reproductive behavior, hostility, and pain.

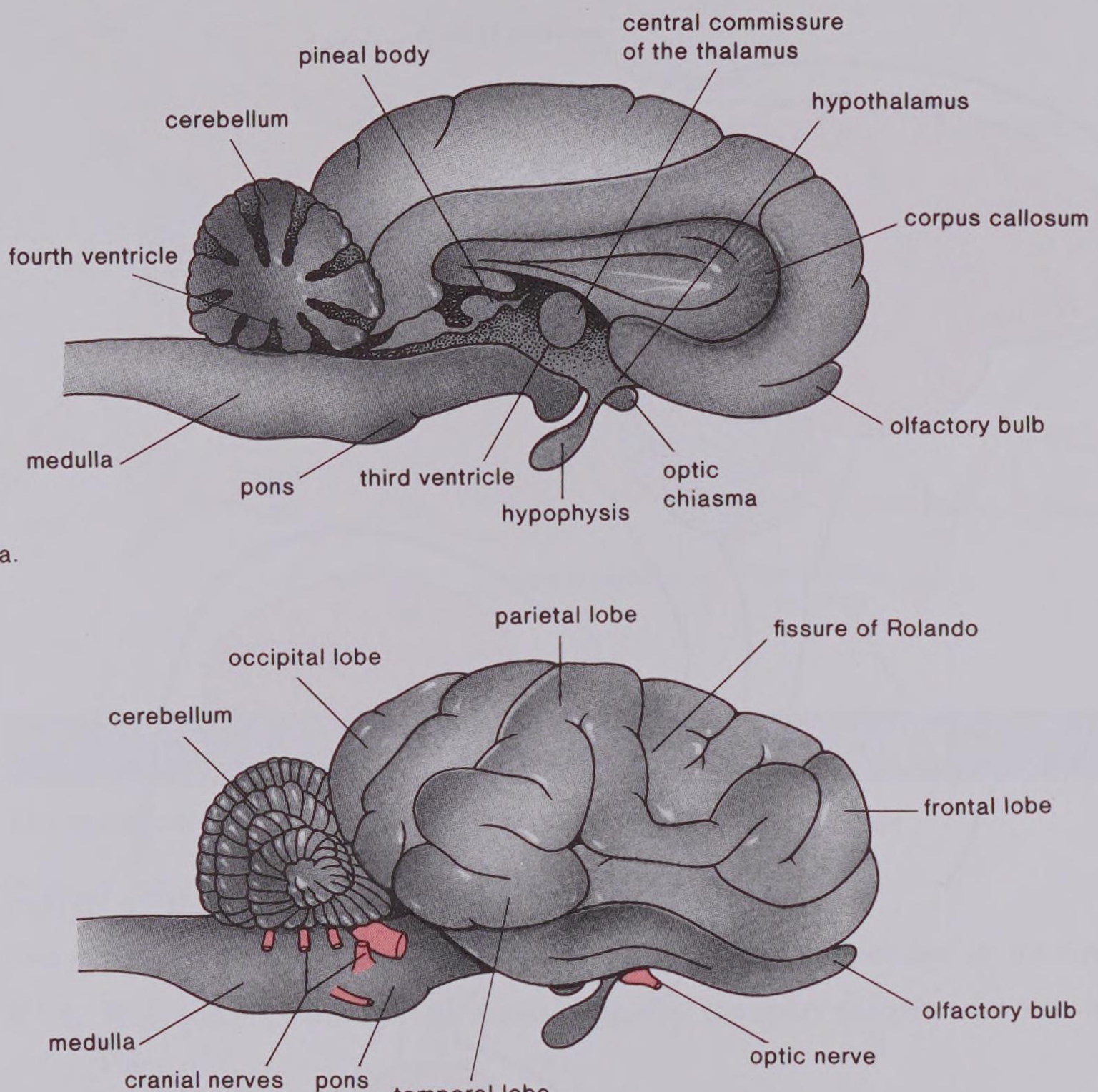
Thalamus

The *thalamus* consists of 2 lobes that are connected by the *central commissure.* The thalamus is a relay station for impulses carried between lower brain centers and the cerebrum. It is the highest portion of the brain to receive sensory impulses, and it apparently controls which impulses are passed on to the cerebrum. For this reason it is sometimes called the "gatekeeper to the cerebrum."

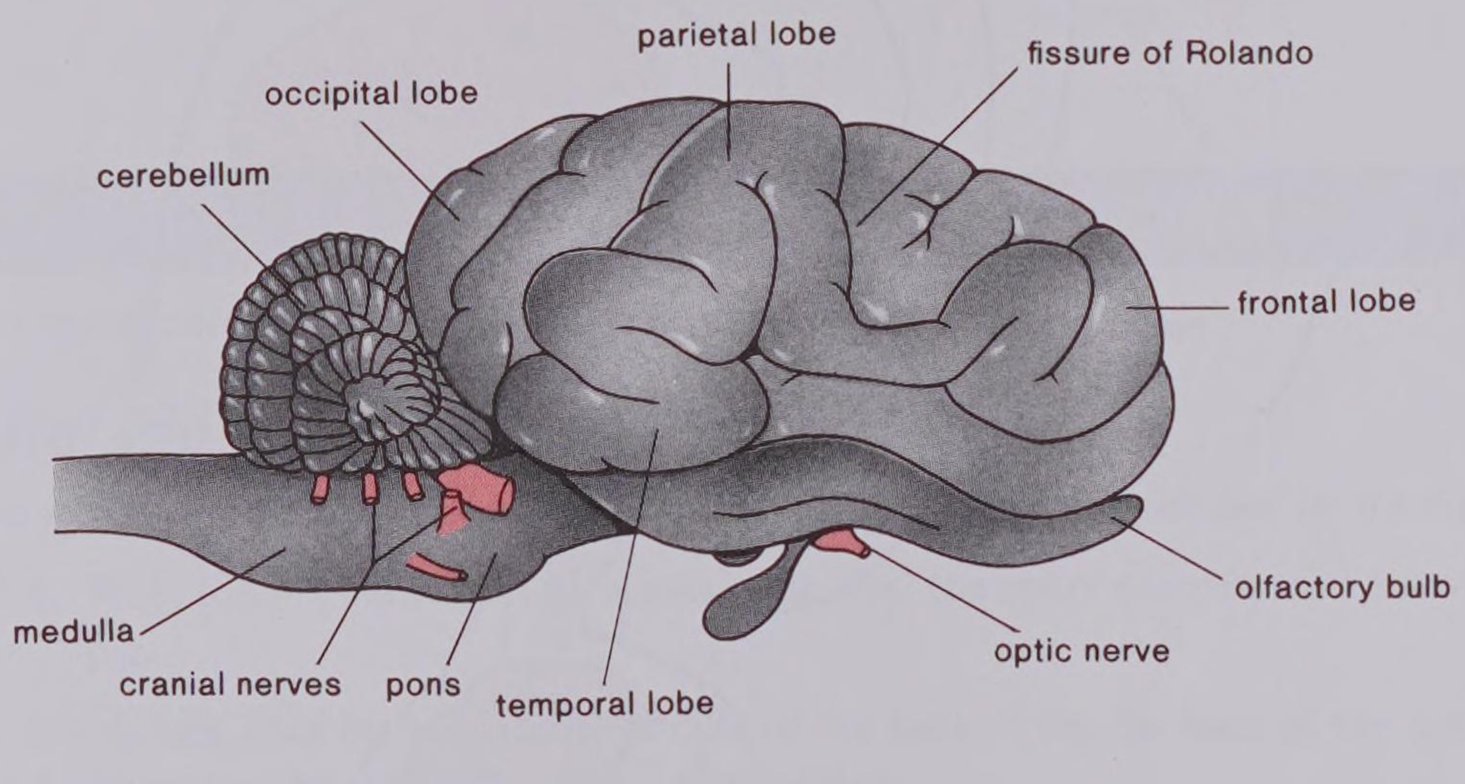
Cerebrum

The *cerebrum* is the most well-developed area of the brain. It is divided into the right and left *cerebral hemispheres,* which are joined by the *corpus callosum,* a broad sheet of white matter. A comparative study of vertebrates indicates a progressive increase in the size of the cerebrum from fish to humans (fig. 27.3). In fish and amphibians, the cerebrum largely has an olfactory function, but in reptiles there is a portion of the cerebrum that receives information from other parts of the brain and coordinates sensory data and motor functions. It is this outer portion of the cerebrum, called the *cortex,* that is highly convoluted in mammals, especially humans.

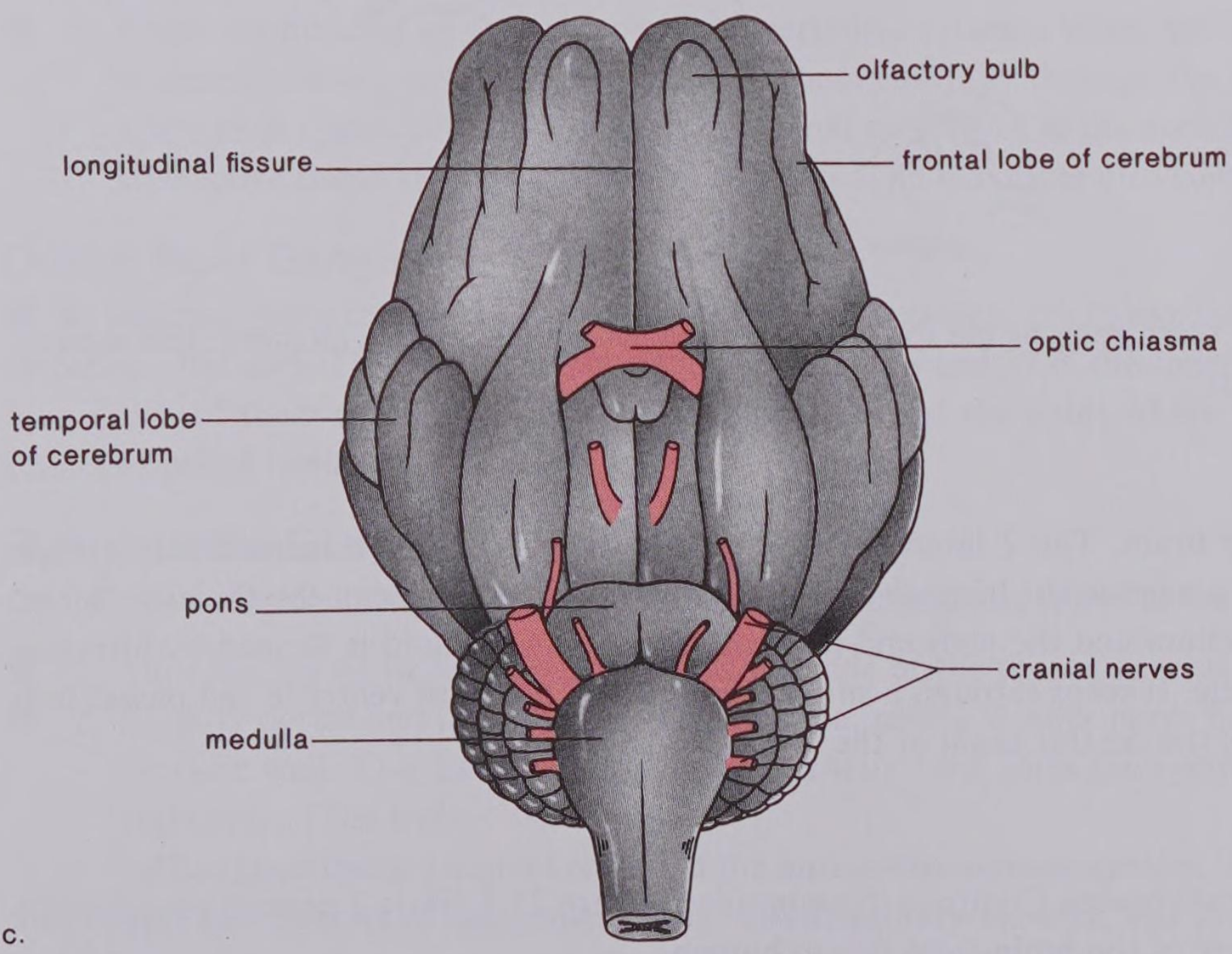
Figure 27.2 Sheep brain. ***a.*** Longitudinal cut. ***b.*** Lateral view. ***c.*** Ventral view.



a.

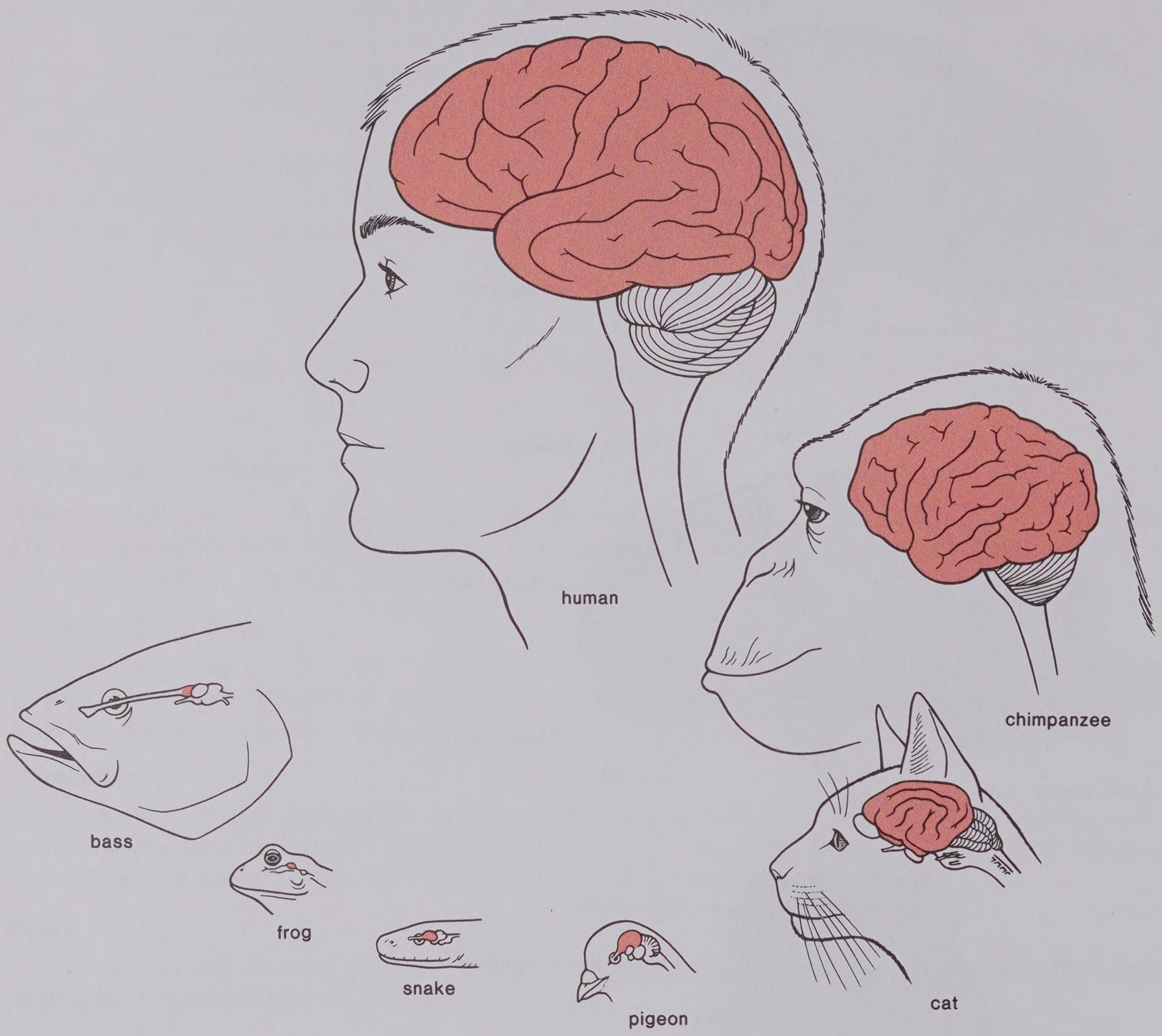


b.



c.

Figure 27.3 Comparison of brain sizes among vertebrates. Notice the increase in size of the cerebrum from fish to human.



The cerebrum is the portion of the brain responsible for consciousness. Intelligence, will, memory, and sensory perception are the functions of the cerebrum.

Ventricles

There are 4 *ventricles* (cavities) within the brain. The 2 lateral ventricles (first and second) are located one in each cerebral hemisphere. The third ventricle is a narrow slit beneath the first 2 ventricles and between the thalamic lobes. The fourth ventricle lies between the cerebellum and the pons and medulla. Cerebrospinal fluid is formed by filtration from a capillary mass in each lateral ventricle. It seeps through a small aperture into the third ventricle and passes into the fourth ventricle by a duct; it then enters the central canal of the spinal cord.

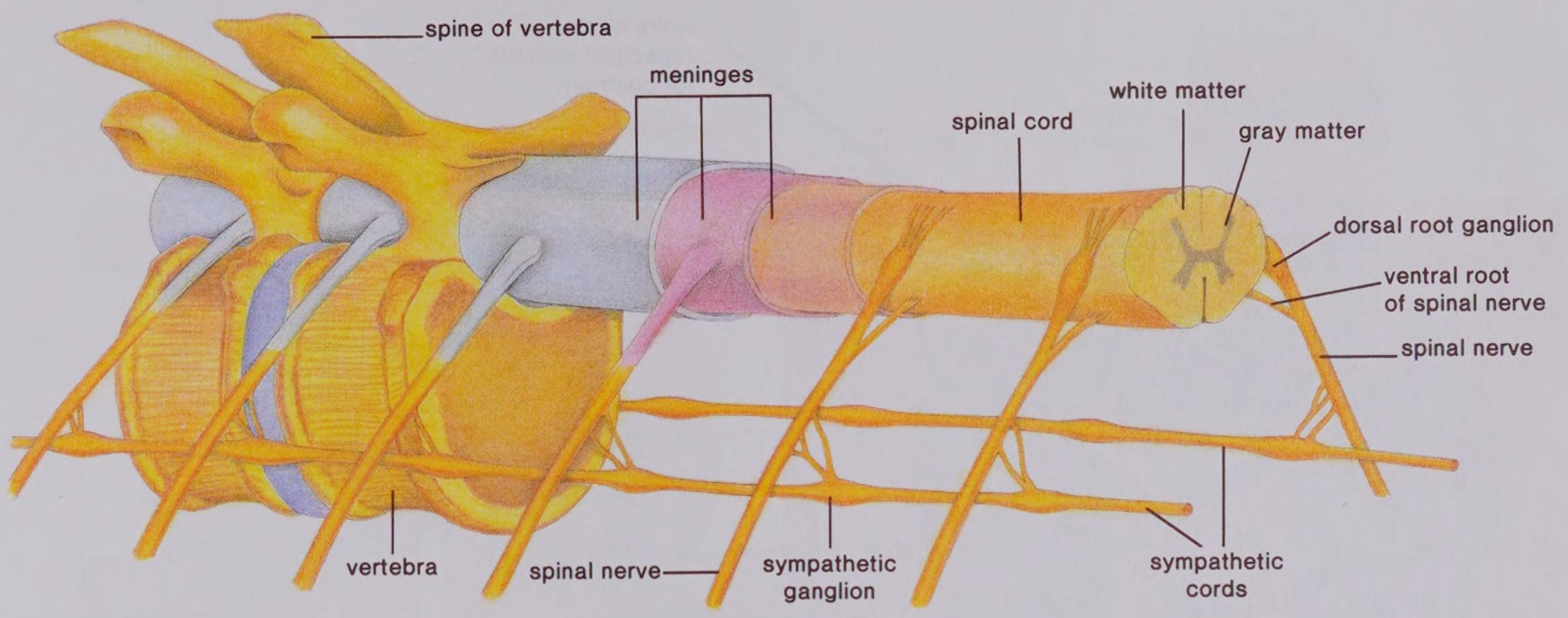
Vertebrate Brains

If available, examine models of other vertebrate brains. Compare these brains to figure 27.3. State 2 general observations you can make about a change in the structure of the brain from fish to human.

1. _____

2. _____

Figure 27.4 Vertebra and spinal cord.



Spinal Cord

The spinal cord is also part of the central nervous system.

Fetal Pig Dissection

The spinal cord is protected along its entire length by the bony arches on the dorsal part of each vertebra (fig. 27.4).

S 1. With the pig resting on its ventral side, *feel* the spiny extensions of the vertebrae by palpating the back of the pig.

S 2. Make a long *incision* in the middle of the back, from the back of the skull to the base of the tail.

S 3. *Separate* the skin and move it to the sides.

S 4. *Clear* the muscles on the surface of the vertebral column. When the spiny processes (extensions) of the vertebrae are exposed, *clip* the tips, split them carefully through the middle, and spread the bony structures apart to the sides in order to reveal the spinal cord lying in the vertebral canal. The cord is surrounded by connective tissue sheaths known as *meninges*. The meninges also cover the brain.

Dorsal Root Ganglia

S By clearing away the muscles close to the vertebral column, try to *expose* small whitish masses associated with each vertebra—the *dorsal root ganglia.* As seen in figures 27.4 and 27.6, the spinal nerves that arise from the cord are formed by a union of their *dorsal* and *ventral roots*. Just beyond the point where the roots unite, a spinal nerve divides into branches called *rami.*

Sympathetic Ganglia

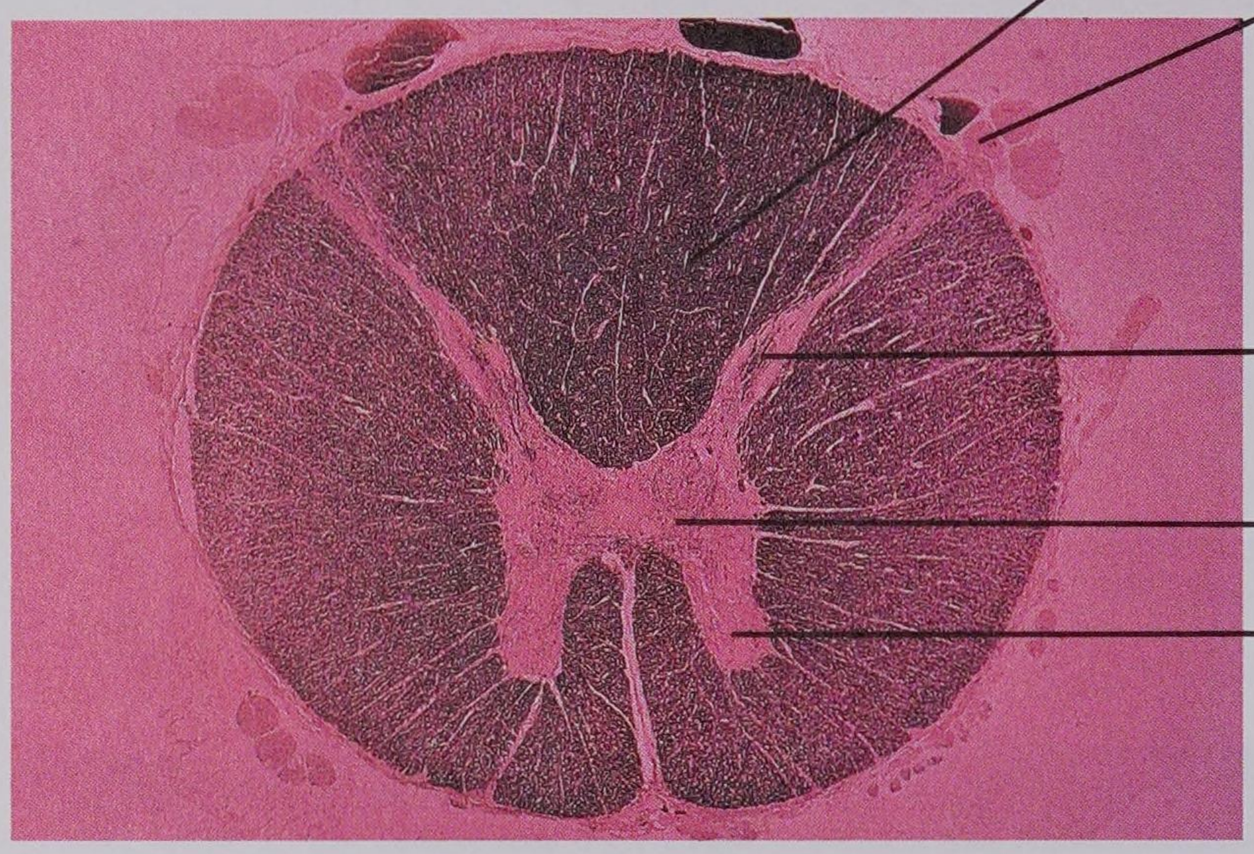
One of the branches from the spinal nerves carries sympathetic fibers to the *sympathetic ganglia.*

S 1. Turn the pig on its back; pull open the left side of the thorax as wide as you can.

S 2. Slightly dorsal and lateral to the dorsal aorta, note a slender nerve band extending longitudinally against the thoracic wall. This band, called the *sympathetic cord,* joins the sympathetic ganglia. Another cord lies on the right side of the body.

The sympathetic ganglia are part of the *autonomic nervous system.* This system consists of special motor neurons that supply the muscles of the heart, blood vessels, urinary bladder, and gut wall, as well as most of the glands of the body. The autonomic nervous system actually consists of 2 divisions—*sympathetic* and *parasympathetic.* Most organs supplied by it receive nerve fibers from both divisions; the 2 types of fibers carry impulses that have antagonistic effects upon the organs. The *vagus nerve,* a cranial nerve, is a part of the parasympathetic system.

Figure 27.5 Enlargement of spinal cord. Note the butterfly shape of the gray matter.



Spinal Cord Microscopic Examination

Ⓢ 1. Examine a cross section slide of the spinal cord under the lowest magnification available. You may use a binocular dissecting microscope or the lowest magnification of your binocular microscope. For example, some microscopes are equipped with a very short scanning objective that enlarges about 3.5×, with a total magnification of 35×. If neither of these alternatives is available, then *observe* the slide against a white background, using the naked eye.

Ⓢ 2. After viewing the slide in one of these ways, *examine* it using 100× magnification.

Ⓢ 3. Position the slide as in figure 27.5 and *identify* the parts shown.

Gray and White Matter

The cord is composed of *gray matter* and *white matter*. The gray matter is easily recognized because it picks up more dye in the staining process.

Ⓢ Notice that the gray matter forms a butterfly shape. The *dorsal horns* are the upper extensions and the *ventral horns* are the lower extensions of the gray matter that account for the butterfly shape.

In some slides you may also be able to see groups of cells that form the *dorsal root ganglia* and connect with the dorsal horns.

Ⓢ In the ventral horns, look for the triangular-shaped *motor neurons*; some may be cut in such a plane that part of the *axon* and *dendrites* may be seen connected to the *cell bodies*. Figure 27.5 may help you with your examination.

After examining figure 27.5, can you determine what would cause certain portions of the cord to appear white? _____

_____

to appear gray? _____

Spinal Nerves

Figure 27.6*a* shows the spinal cord and spinal nerve.

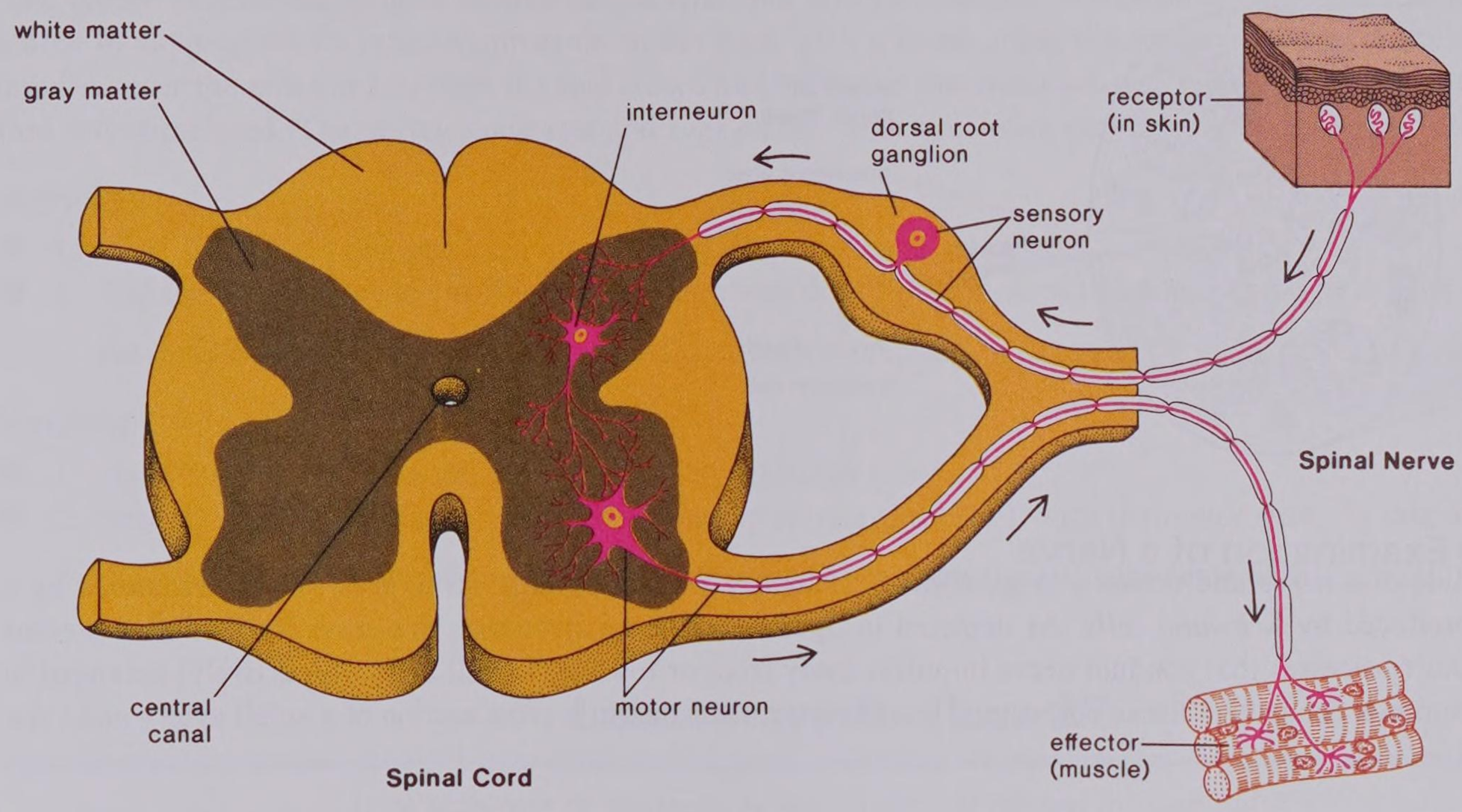
Ⓢ Note the portion of the diagram that represents the cord and the portion that represents the nerve. Whereas the spinal cord is in the *central nervous system* (CNS), spinal nerves are in the *peripheral nervous system.*

1. What does peripheral mean? _____
2. What 2 types of neurons have extensions in the spinal nerve?

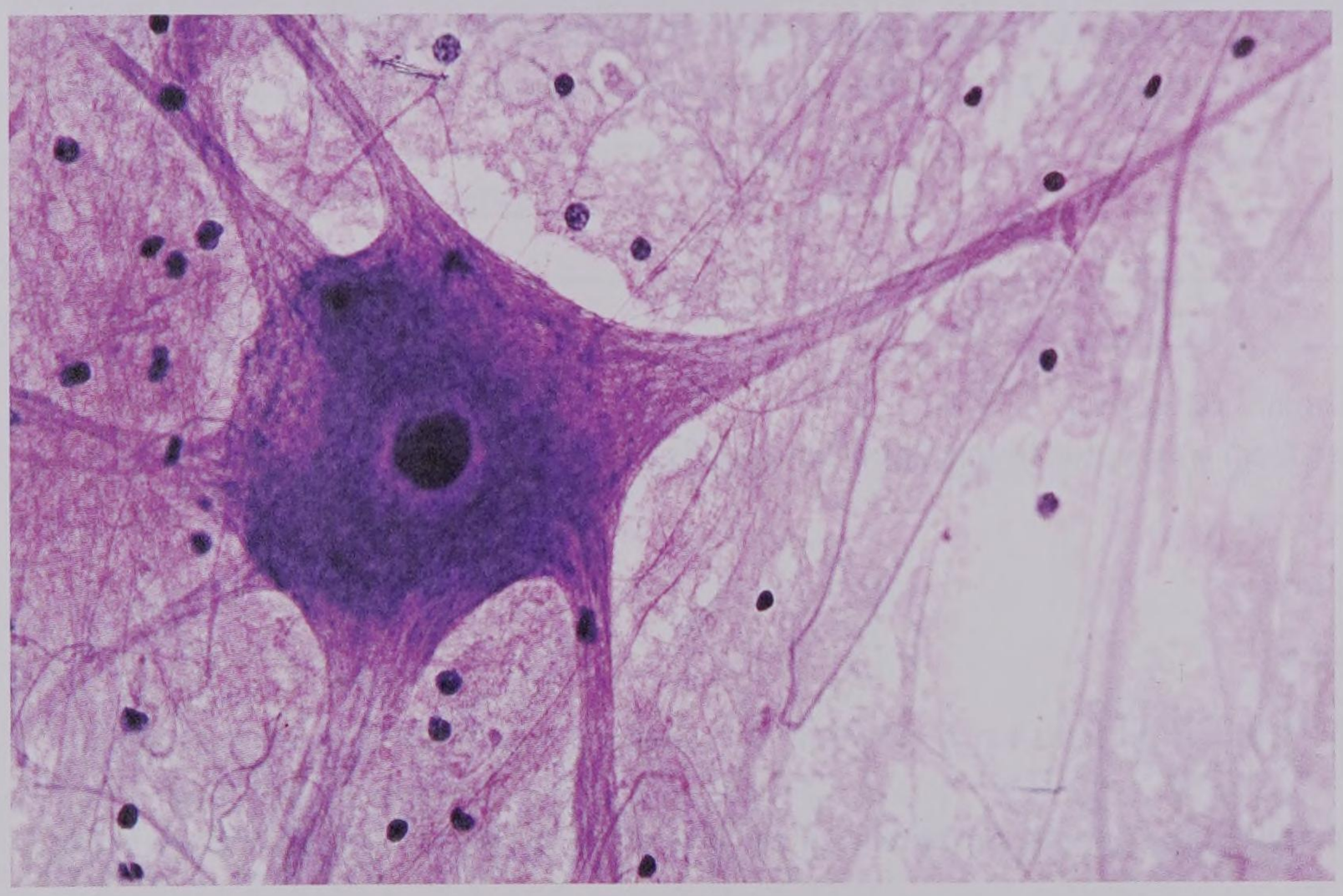
   a. _____

   b. _____

Figure 27.6 ***a.*** Spinal cord and spinal nerve. The arrows mark the path of a reflex arc. ***b.*** Photomicrograph of neurons from spinal cord.



a.



b.

3. What portion of the sensory neuron is in the nerve? _____

   Where is the cell body of the sensory neuron? _____

   Where is the axon of the sensory neuron? _____

   What is the function of the sensory neuron? _____

4. What portion of the motor neuron is in the nerve? _____

   Where is the cell body of the motor neuron? _____

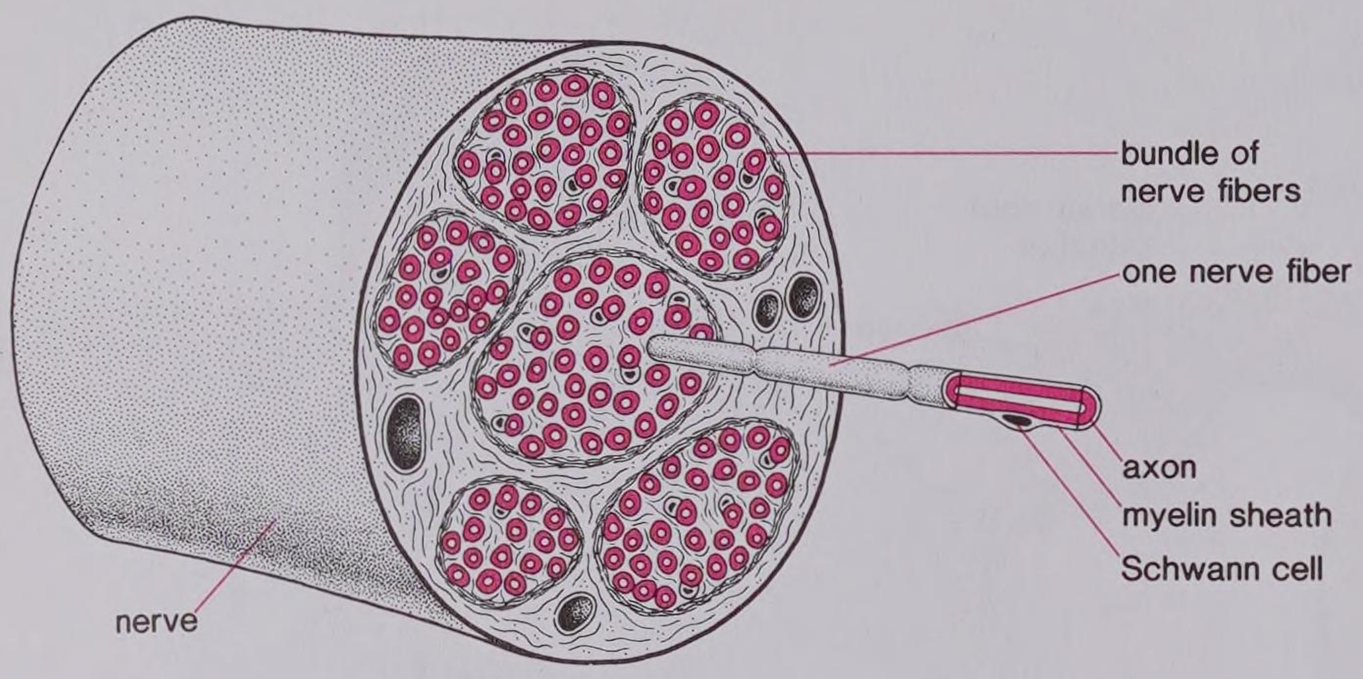
   Where are the dendrites of the motor neuron? _____

   What is the function of the motor neuron? _____

5. Why are spinal nerves called mixed nerves? _____

   _____

Figure 27.7 Cross section of a nerve showing that a single nerve has many fibers.



Microscopic Examination of a Nerve

Examine a slide of a *nerve* and notice a large number of lightly stained circular structures, some surrounded by a *myelin sheath* produced by *Schwann cells*. As depicted in figure 27.7, these structures are cross sections of numerous *axons* or *dendrites* (processes that conduct nerve impulses away from or toward the cell body, respectively) arranged in bundles with a common connective tissue covering. The slide may also contain a cross section of a small artery and vein.

Spinal Reflex

A spinal reflex action requires 3 neurons. Using figure 27.6 as a reference, indicate what function in the arc is performed by the

1. sensory neuron _____
2. interneuron _____
3. motor neuron _____

These 3 neurons form the so-called spinal reflex arc.

Trace here the path of an impulse from a receptor to the cerebrum (see fig. 27.6).

1. _____ receptor
2. _____ neuron
3. _____ neuron
4. _____ (portion of brain)
5. cerebrum

Bundles of long fibers of interneurons that take impulses to the brain are called *tracts*. What is the difference between a tract and a nerve? _____

Which portion of the cord contains tracts (gray or white matter)? _____

What portion of figure 27.6 is the central nervous system? _____

What portion of figure 27.6 is in the peripheral nervous system? _____

Human Reflexes

A reflex is a predictable response to a given stimulus. Of the many reflexes that occur in the body, only tendon reflexes are investigated here.

Tendon Reflexes

Two tendon reflexes easily done involve the *patellar* and *Achilles* tendons. When these tendons are mechanically stimulated by tapping with a reflex hammer or, in our case, with a meter stick, the sensory neurons in the tendon transmit impulses over the reflex arc through the spinal cord and back over the motor neurons to the muscle attached to the tendon that was stimulated. The muscle contracts and tugs on the tendon, causing movement of a bone opposite the joint.

Achilles

1. Have the subject rest one knee on a chair so that the leg is extended.
2. *Tap* the Achilles tendon at the ankle. Which way does the foot move? (Toward the knee is *flexion;* away from the knee is *extension.*) _____

Patellar

1. Have the subject sit on the table so that the leg hangs free.
2. Sharply *tap* the patellar tendon, just below the patella (kneecap), with the meter stick. In this relaxed state, does the leg move toward the buttocks (flexion) or away from the buttocks (extension)? _____

Laboratory Review 27

_____ 1. Which part of the brain is most highly developed in mammals?

_____ 2. What is the function of the cerebellum?

_____ 3. Where will you find the cell bodies of most neurons?

_____ 4. What structures protect the brain and spinal cord?

5. What is the difference between a nerve and a neuron?

6. How does the central nervous system differ from the peripheral nervous system?

7. If the dorsal root ganglia are destroyed, can impulses reach the spinal cord? Why or why not?

8. How many neurons are involved in the simplest reflex arc? What is the function of each?

References

Curtis, Dwayne H. 1974. *Human Physiology Laboratory Experiments.* Dubuque, IA: Kendall/Hunt Publishing Company.

Jakowska, S. 1973. *SICC Laboratory Manual for Biology 101.* Dubuque, IA: Kendall/Hunt Publishing Company.

Laboratory

28

Senses

Learning Objectives

Students should be able to

1. identify the parts of the eye, ear, and skin mentioned and state a function for each part;
2. describe the experiments that make up this laboratory session and explain their significance.

Introduction

Each type of sense organ is sensitive to a particular type of environmental stimuli, as indicated in table 28.1. Sense organs are called *receptors* because they receive stimuli and thereafter generate nerve impulses. Eventually these nerve impulses reach the brain, where the phenomenon sensation occurs. The brain interprets the world around us and is dependent on the sense organs for the proper input. Since the sense organs are limited in their ability to respond to the environment, we may be misled by them.

Table 28.1 Sense Receptors

Sense Organs	Sense	Stimulus	Types
General			
Temperature	Hot–cold	Heat flow	Thermoreceptor
Touch	Touch	Mechanical displacement of tissue	Mechanoreceptor
Pressure	Pressure	Mechanical displacement of tissue	Mechanoreceptor
Pain	Pain	Tissue damage	Chemoreceptor
Proprioceptors	Limb placement	Mechanical displacement	Mechanoreceptor
Special			
Eye	Sight	Light	Photoreceptor
Ear	Hearing	Sound waves	Mechanoreceptor
	Balance	Mechanical displacement	Mechanoreceptor
Taste buds	Taste	Chemicals	Chemoreceptor
Olfactory cells	Smell	Chemicals	Chemoreceptor

Eyes

Human Eye

S With the help of figure 28.1*a*, identify the following *structures* when examining an eye model. With the help of table 28.2, give a *function* for each structure in the space provided.

Sclera _____

Cornea _____

Choroid _____

Retina _____

Fovea _____

Lens _____

Ciliary body _____

Iris _____

Pupil _____

Optic nerve _____

Which of the structures listed aid in refracting and focusing the light rays? _____

_____

Which of those structures contains the receptors for sight? _____

What are the receptors for sight? _____

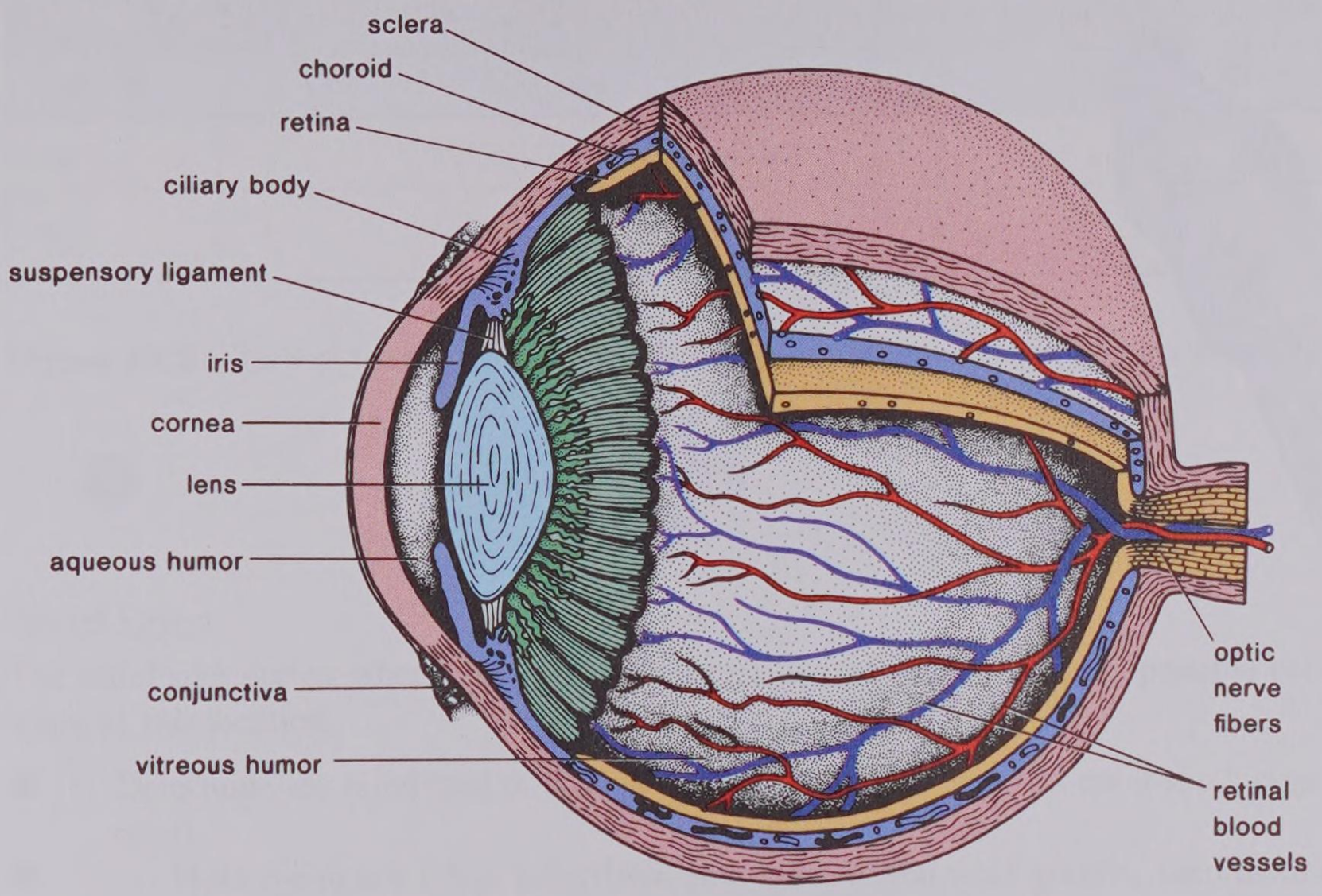
Which of the structures listed transmits nerve impulses to the brain? _____

Table 28.2 Name and Function of Parts of the Human Eye

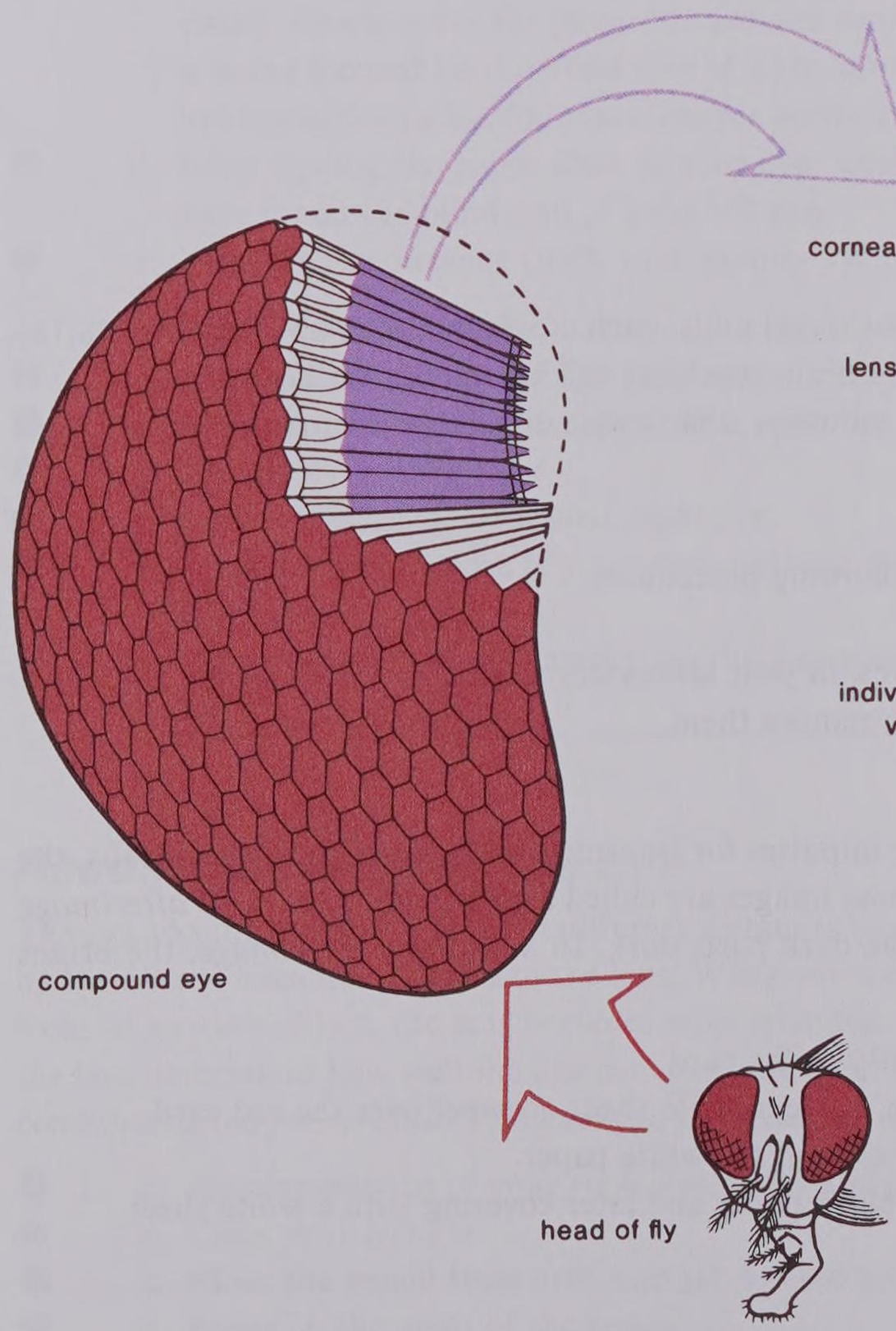
Part	Function
Lens	Refraction and focusing
Iris	Regulates light entrance
Pupil	Admits light
Choroid	Absorbs stray light
Sclera	Protection
Cornea	Refraction of light
Humors	Refraction of light
Ciliary body	Holds lens in place
Retina	Contains receptors (rods and cones)
Rods	Black-and-white vision
Cones	Color vision
Optic nerve	Transmits impulse
Fovea	Contains cones in retina
Ciliary muscle	Accommodation

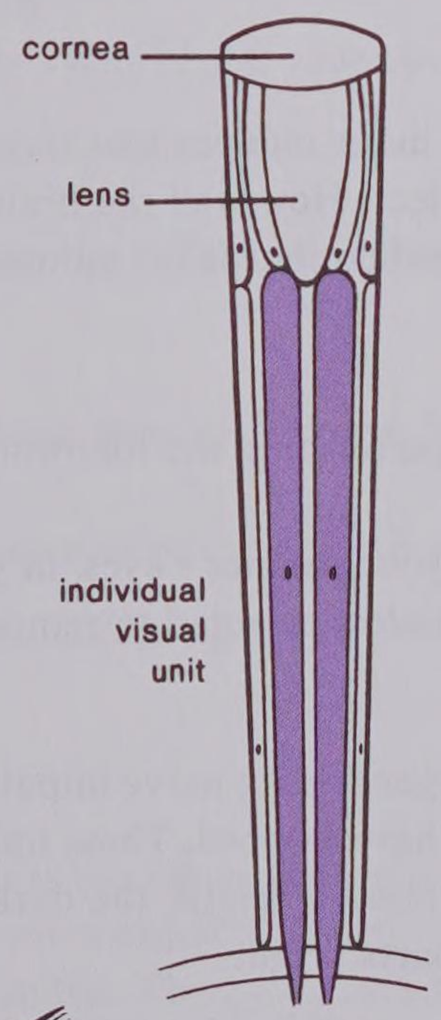
S Notice that humans, like the squid (fig. 28.2), have a *camera type* of eye. There is a single lens that focuses an image of the visual field on the photoreceptors, which are closely packed together.

Figure 28.1 ***a.*** Human eye. Note the single lens. ***b.*** Compound eye of insects. Note the numerous lenses.



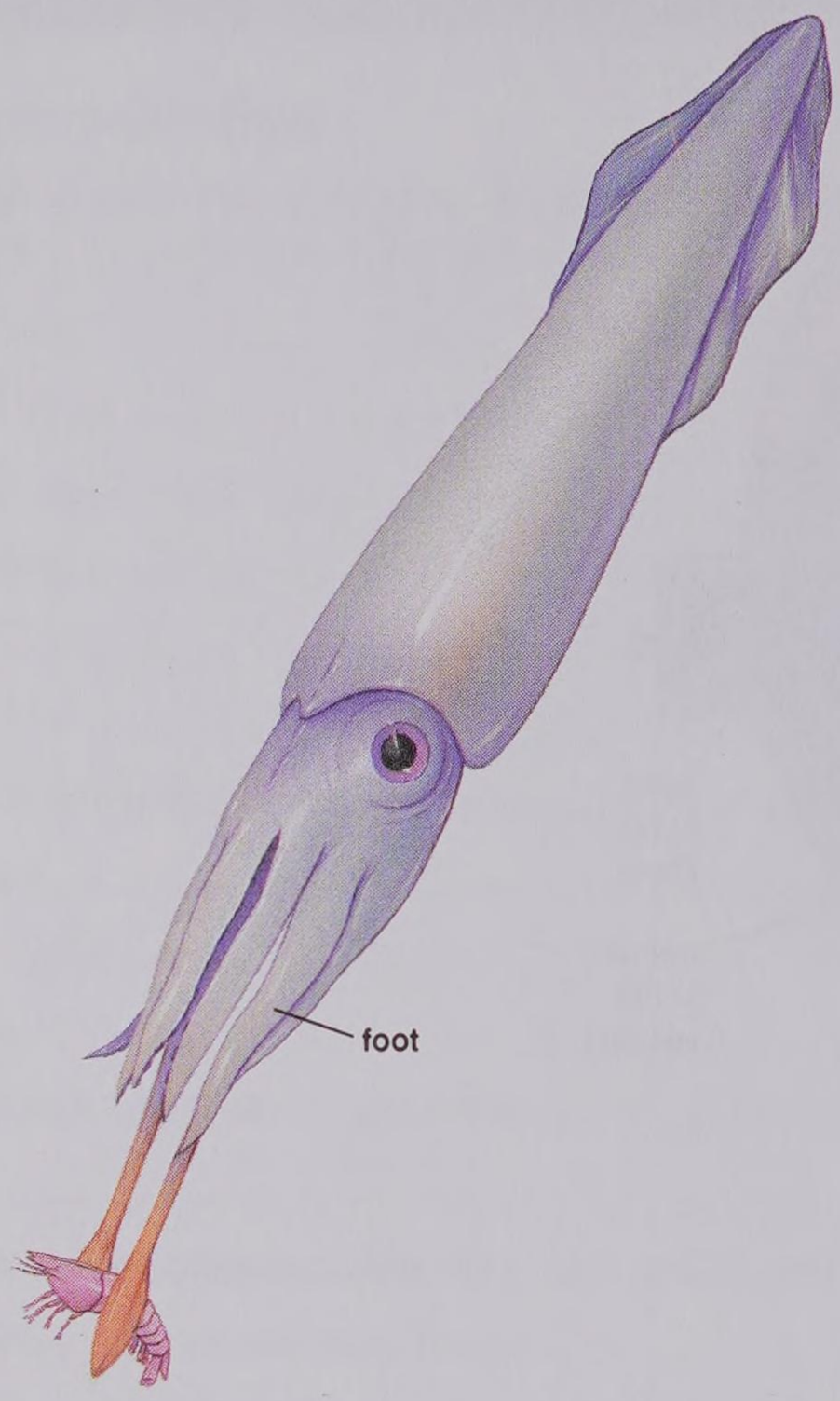
a.





b.

Figure 28.2 The squid, a molluskan invertebrate, has a camera-type eye similar to those of vertebrates.



Arthropod Eye

Arthropods have compound eyes composed of many independent visual units, each of which has its own lens (fig. 28.1*b*). Each unit "sees" a separate portion of the object. How well the brain combines this information is not known.

Ⓓ Ⓢ Examine the *grasshopper eye* demonstration that is set up under a binocular dissecting microscope.

Experimental Procedures

Ⓢ 1. Select a laboratory *partner* to help in performing the following procedures.
Ⓢ 2. Perform the test on *both* partners.
Ⓢ 3. Enter the data for your *own* eyes, not your partner's eyes, in your laboratory manual.
Ⓢ 4. If you wear *eyeglasses*, keep them *on* unless directed to remove them.

Afterimages

We see images because the rods and cones are generating nerve impulses for transmission to the brain. Occasionally, the brain retains an image even after the impulses have stopped. These images are called afterimages. A *positive afterimage* is one in which the bright parts of the object remain bright, the dark parts dark. In a *negative afterimage,* the bright parts of the object appear dark and the dark parts bright.

Ⓢ 1. Center a red card on a black sheet of paper. Stare intently at the card.
Ⓢ 2. After 30 seconds or longer and without shifting the eyes, place a white sheet of paper over the red card.
Ⓢ 3. Describe in table 28.3 the color of the image that "appears" on the white paper.
Ⓢ 4. Repeat by placing green, blue, and yellow cards on the black paper and later covering with a white sheet.

Table 28.3 Afterimages			
Color	**Color of Afterimage**	**Color**	**Color of Afterimage**
Red		Yellow	
Blue		Green	

Figure 28.3 To test for the blind spot, use these symbols and the directions provided by the text.

Blind Spot

The blind spot occurs where the *optic nerves* exit the retina. No vision is possible because of the absence of rods and cones at this location.

1. Determine the blind spot of the left eye. In figure 28.3 you will see a small cross and a circle a couple of inches apart.
   a. Hold the figure 1 foot away from your eyes. If you wear glasses, keep them on.
   b. Close your *right eye only.*
   c. Stare at the *cross only* with your *left* eye. You should also be able to see the circle in the same field of vision. Slowly move the paper toward you until the circle disappears! At that point, the light from the circle is being focused on the blind spot of your left eye. The blind spot is the area of the retina that contains no light-sensitive cells. You therefore see nothing.
   d. Keep moving the paper close to your eyes until the circle reappears. Since only your left eye is open, you have found the blind spot of your left eye.
   e. Measure the distance (with your partner's help) from your eye to the paper when the circle first disappears.

   What is the distance? _____ left eye
2. Determine the blind spot of the right eye.
   a. Repeat the procedure just described, with the following modifications.
   b. Close only your *left eye.*
   c. Look at the *circle* with your *right eye.*
   d. Move the paper close to your eyes until the cross disappears. You have now found the blind spot of your *right* eye.
   e. Again measure the distance from the paper to your right eye when the cross first disappears.

   What is the distance? _____ right eye. Is the distance the same for both eyes? _____

Accommodation of the Eye

The eye accommodates for focus at different distances by changing the shape of the *crystalline lens*. The lens is controlled by the *ciliary muscles* attached to the lens. When one looks at a distant object, the lens is in a flattened state. When one looks at a closer object, the lens becomes more rounded. The eye is said to accommodate for distance. The elasticity of the lens determines how well the eye can accommodate. The elasticity of the lens decreases with an increase in age in a condition called *presbyopia*. This causes a progressively increasing farsightedness, the inability to focus on objects near.

1. a. Accommodation of your *right* eye. Hold a pencil by the eraser in your right hand.
   b. Close your *left eye.*
   c. Move the pencil from arm's length toward your *right eye.*
   d. Focus on the point of the pencil.

e. Move the pencil until the *point* becomes out of focus. Measure the distance between the pencil and your right eye.

At what distance can your right eye no longer accommodate for distance? _____

f. If you wear glasses, repeat this experiment without your glasses. Accommodation distance of the right eye without glasses _____ (Contact lens wearers need not make these determinations, and they should write the words contact lens in this blank and in the next section for the left eye.)

2. Accommodation of your left eye.
   a. Repeat the procedure described.
   b. Close your *right eye* and look at the pencil point with your left eye. Determine the accommodation distance of your left eye. _____ If you wear glasses, repeat the same experiment without your glasses. Accommodation distance of the left eye without glasses _____ The "younger" lens can easily accommodate for closer distances. The more elastic the lens, the "younger" the eye. The nearest point at which the pencil point can be clearly seen is called the *near point*.

Age (years)	10	20	30	40	50	60
Near Point (centimeters)	9	10	13	18	50	83

3. How "old" is your right eye? _____

   How "old" is your left eye? _____

   How "old" is your right eye without glasses? _____

   How "old" is your left eye without glasses? _____

   Circle your actual age category (optional). Age category (years): 15–19, 20–29, 30–39, 40–49, 50–59, 60–69+.

Pupillary Reflexes

1. This test must be conducted in a *dimly lit* area.
2. The experimenter faces the subject and looks at the pupils.
3. The subject closes his or her eyes or covers them with the hand for approximately 2 minutes.
4. The subject opens his or her eyes and the experimenter shines a *pen light* into one eye, then the other, and back again.

   What happens to the pupils? _____

5. Next, the subject holds the edge of an *index card* along the nose so as to divide the field of vision. The experimenter tests the *pupillary reflex* of one eye, using the pen light as just described.

   Does a comparable reflex occur in the nonilluminated eye? _____

   Do the pupils adjust independently or together? _____

Superimposition

1. Obtain a *paper tube*, such as a mailing case, about 4 cm in diameter and ½ m long, or roll a sheet of paper into such a tube.
2. Keeping both eyes *open*, hold the tube up to one eye and look at some distant object through the tube.
3. Bring the free hand up in front of the other eye and move this hand back and forth along the tube (fig. 28.4). A hole will appear in the *hand*.
4. Explain with reference to figure 28.5.

_____

_____

Figure 28.4 Method used to demonstrate superimposition.

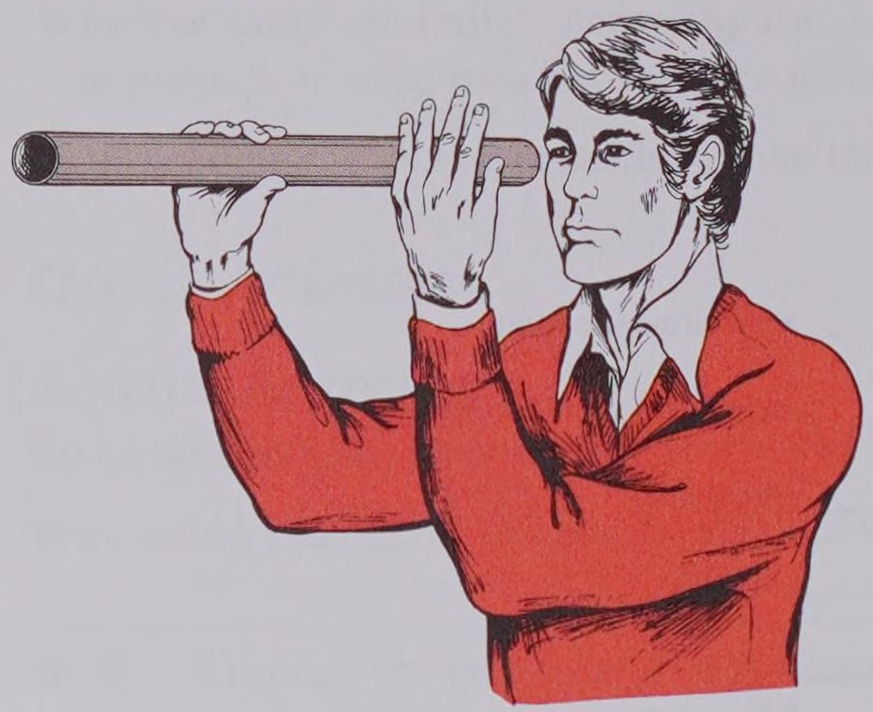
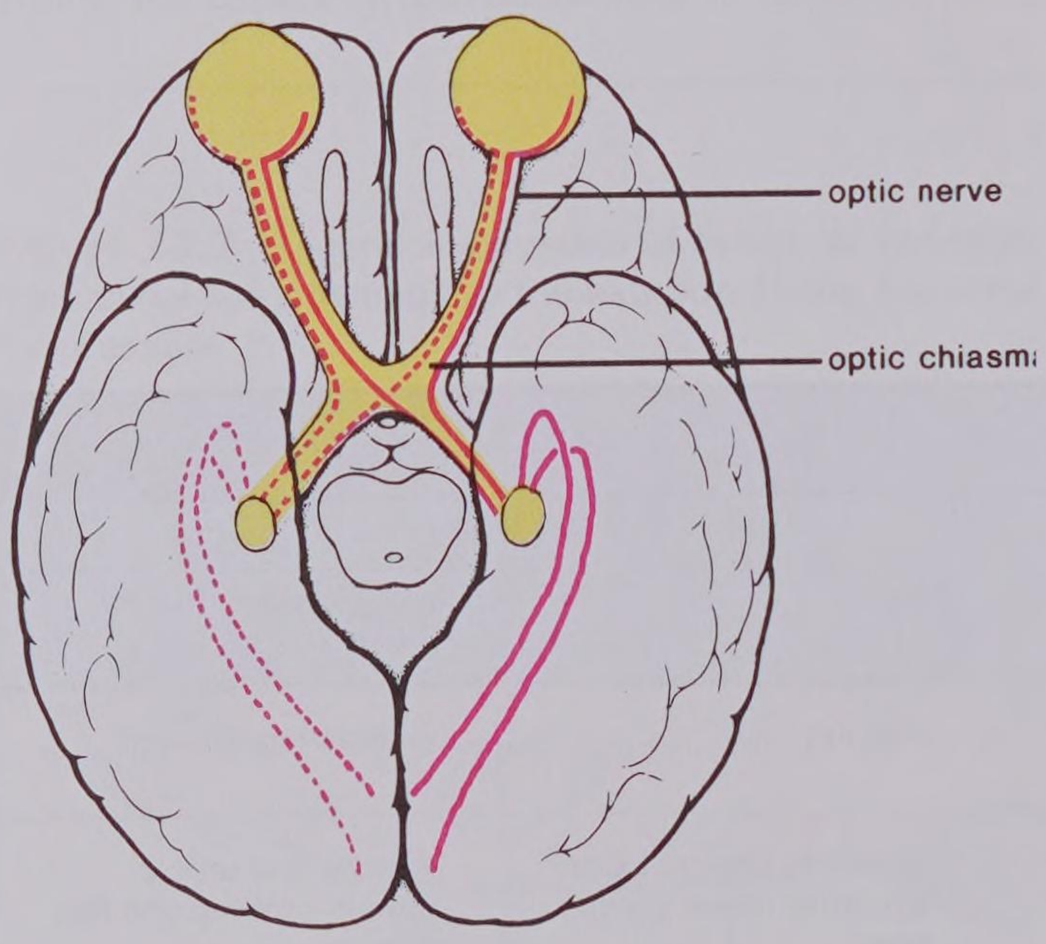


Figure 28.5 Optic chiasma. Both eyes view the same field, but, because of the optic chiasma, both eyes send information about the right field to the left side of the brain and vice versa. If each eye by chance views a different field, the information is mixed in the brain.



Ears

Human Ear

Ⓢ With the help of figure 28.6, study the model of the ear and identify the *outer, middle,* and *inner* ear and their parts, listed following. With the help of table 28.4 give a *function* for each part named.

1. Outer ear

   Pinna _____

   Auditory canal _____
2. Middle ear

   Tympanic membrane (eardrum) _____

   Ossicles (hammer, anvil, stirrup) _____

Figure 28.6 Anatomy of human ear.

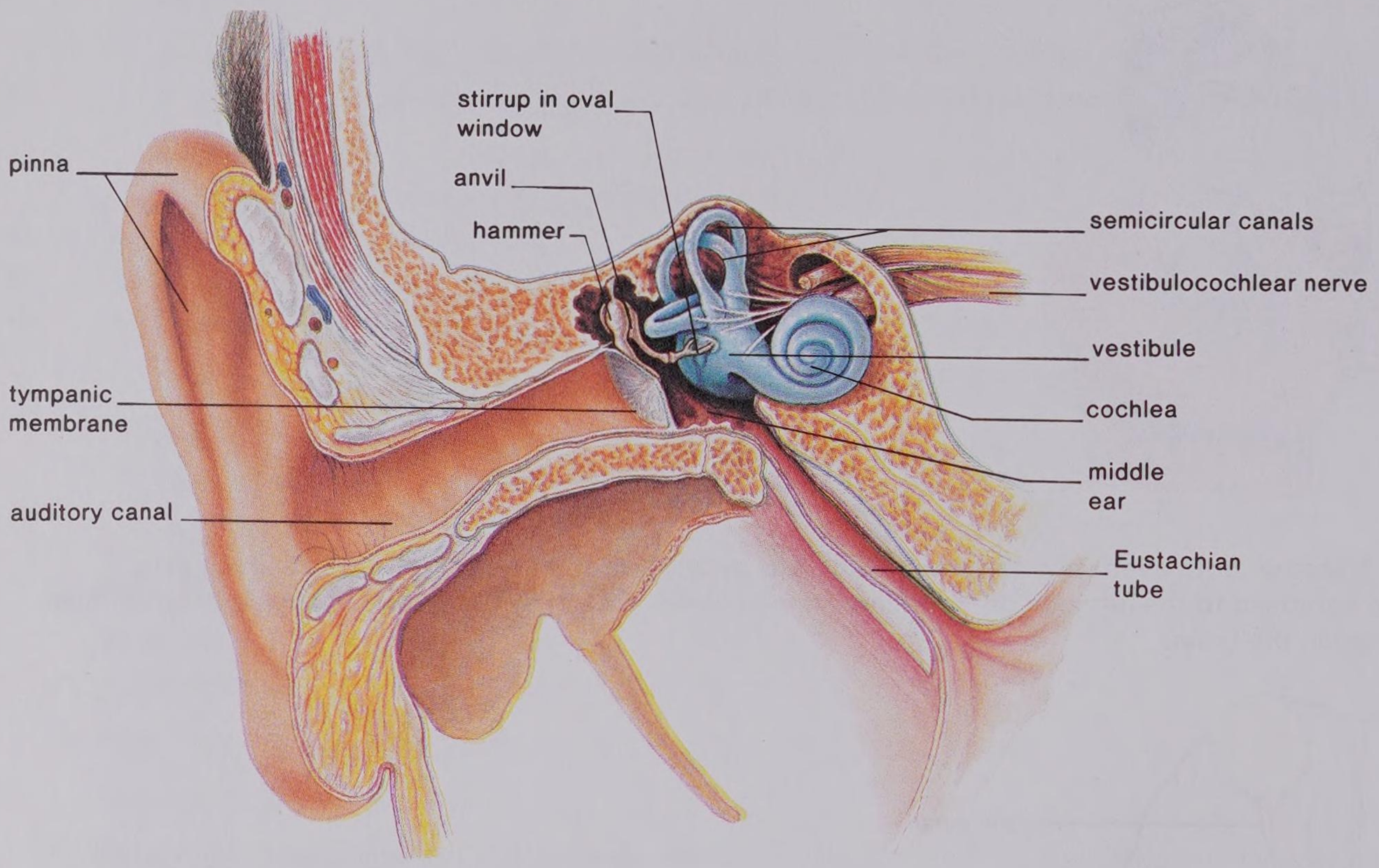


Table 28.4 The Ear

	Outer	Middle	Inner	
Parts			*Cochlea*	*Sacs Plus Semicircular Canals*
Function	Direct sound waves to tympanic membrane	Pick up and amplify sound waves	Hearing	Maintain equilibrium
Anatomy	Pinna Auditory canal	Tympanum cochlea Hammer (malleus) Anvil (incus) Stirrup (stapes)	Contains organ of Corti Auditory nerve starts here	Saccule and utricle contain otoliths and hair cells Semicircular canals contain hair cells in ampullae
Medium	Air	Air	Fluid	Fluid

Path of vibration: Sound waves—vibration of tympanic membrane—vibration of hammer, anvil, and stirrup—vibration of oval window—fluid pressure waves in canals of inner ear lead to stimulation of hair cells (converts pressure waves into nerve impulses and these are conducted to the auditory region of the brain)—bulging of round window (absorbs pressure waves so that resonance does not occur).

3. Inner ear

   Cochlea _____

   Semicircular canals _____

   Auditory nerve _____

Which of these structures transmits sound waves? _____

Which contains the receptors for hearing? _____

What are the receptors of hearing? _____

Which of these transmits a nerve impulse to the brain? _____

The Eustachian tube runs between the middle ear and the throat. It helps equalize the external air pressure with that in the middle ear. Is the Eustachian tube indicated in the model? _____

Other Animals

Aquatic Animals

Do aquatic animals such as fish have external ears? _____

Why would you expect that ears would not be needed by aquatic animals? _____

_____

D **S** Examine the preserved *fish* demonstration and figure 28.7. Notice in figure 28.7 that fish have a *lateral line*, a series of mechanoreceptors that detect water currents and pressure waves from nearby objects. Although these receptors are similar to those in the saccule and utricle of the human ear, the function of the lateral line is to supplement vision.

Terrestrial Animals

Would you expect terrestrial animals to have ears? _____

_____

Figure 28.7 Lateral line system of fishes. ***a.*** Location of the system is shown. ***b.*** Longitudinal section of system. A main canal has openings to the exterior. Lining the canal are hair cells (embedded in cupulae) that act as sense receptors for pressure.

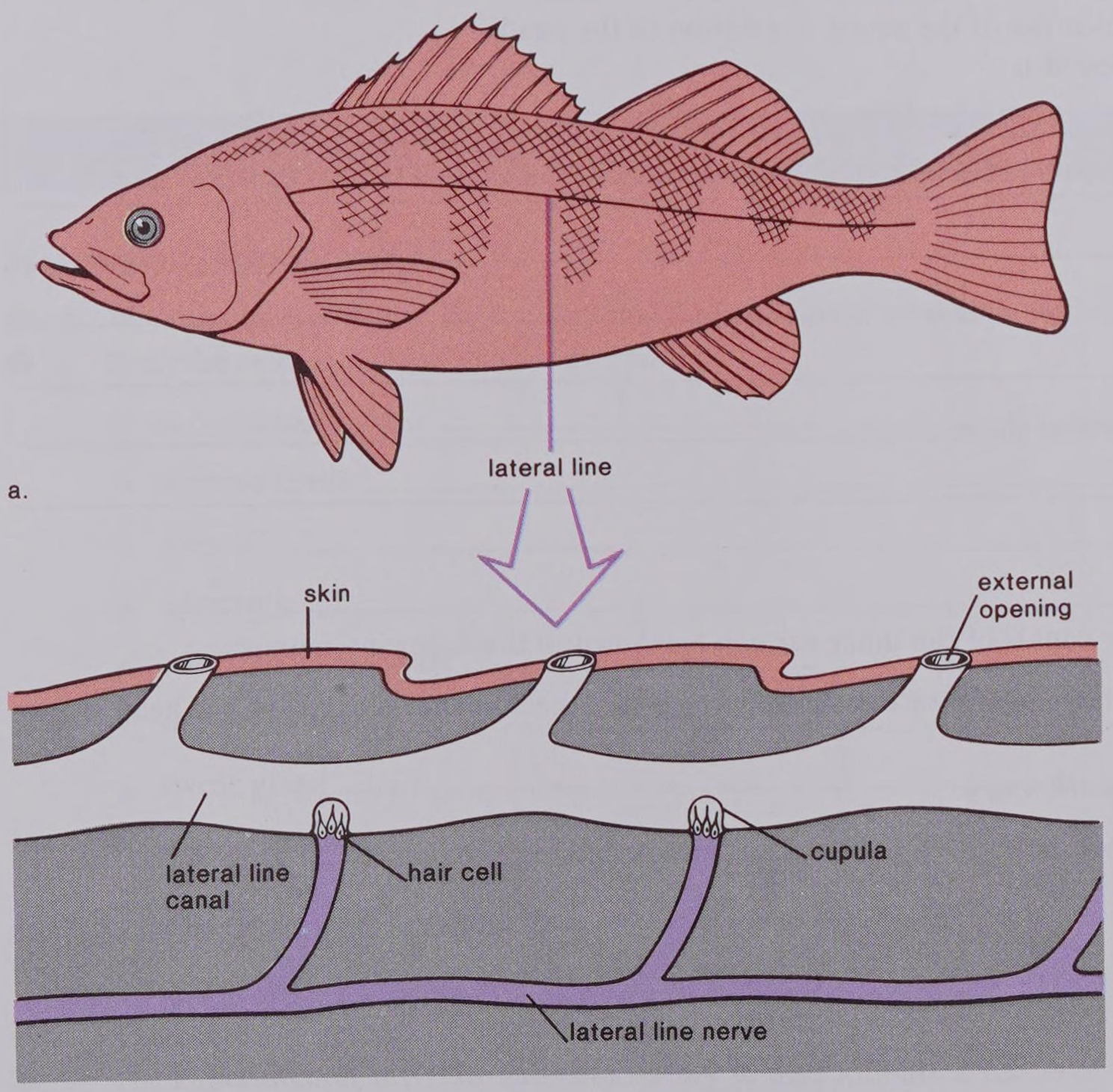
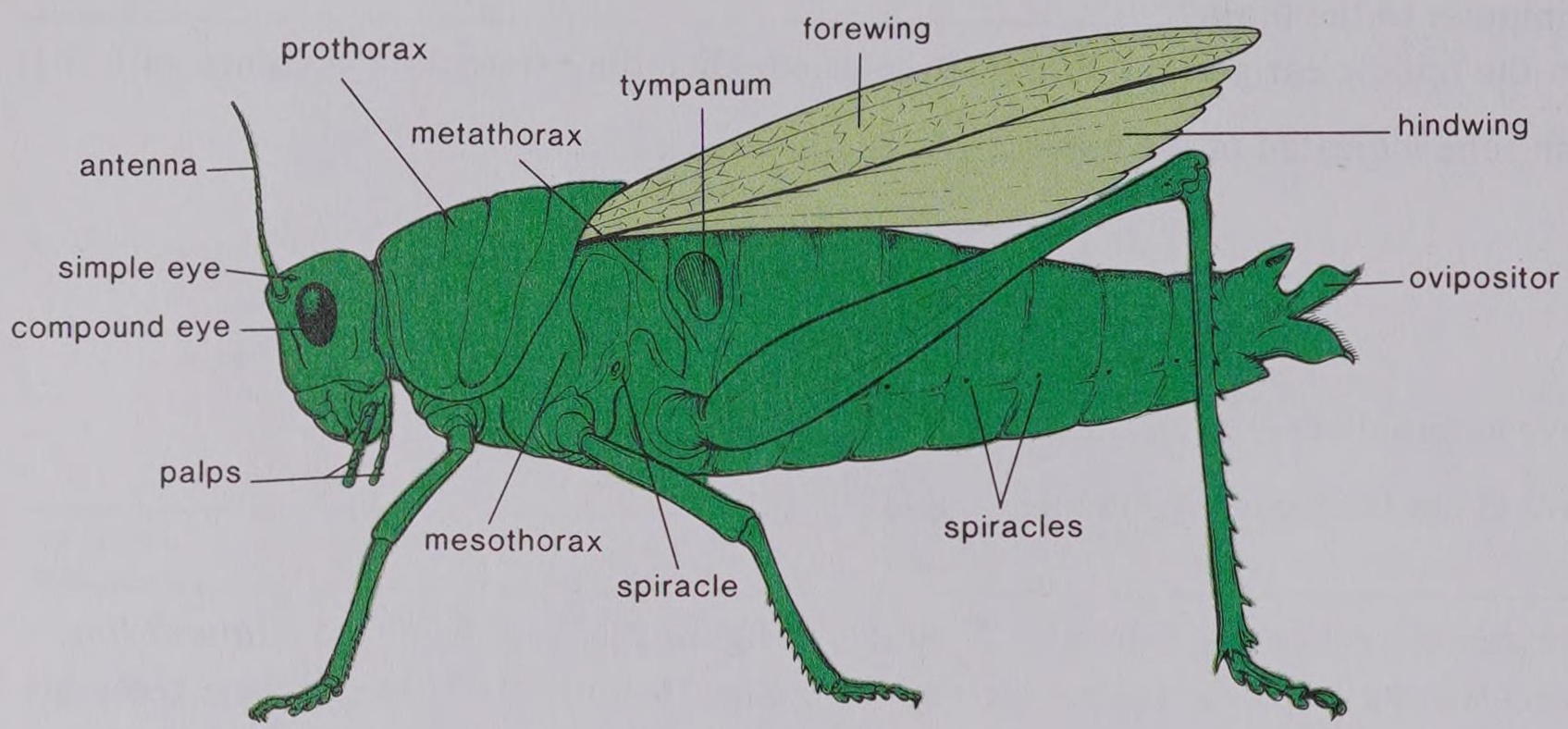


Figure 28.8 External anatomy of grasshopper, side view.



D S For example, examine the preserved grasshopper on display and, with the help of figure 28.8, locate the *tympanum*. The tympanum is the sense receptor for sound waves in the grasshopper.

Experimental Procedures

Sound Direction

S 1. A seated subject closes both eyes.
S 2. The experimenter strikes a *tuning fork,* or strikes 2 spoons together.
S 3. Ask the subject to give the exact *location* of the sound in relation to the head.
S 4. Record the perceptions when the sound is

a. directly below and behind the head: _____

b. directly behind the head: _____

c. directly above the head: _____

d. directly in front of the face: _____

e. to the side of the head: _____

S 5. Explain why the subject missed some of these. _____

_____

Balance

The control of balance by the semicircular canals of the inner ear can be shown in the following manner.

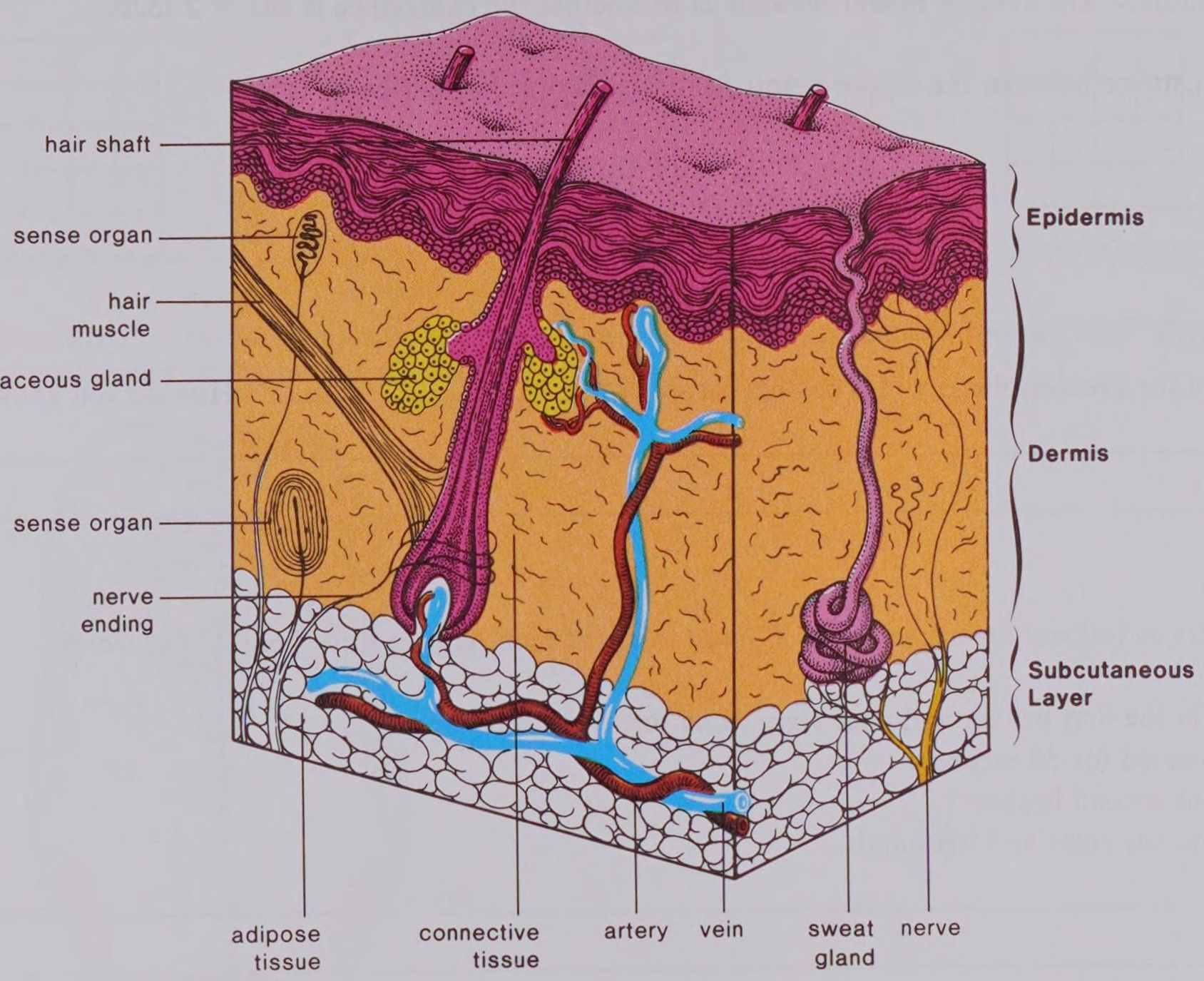
S 1. Have a student sit on a revolving chair; now *turn* the chair slowly while the student holds his or her head on one shoulder or the chest.

S 2. Stop the chair and let the student *walk* away.

How do you explain the student's poor control of balance? _____

_____

Figure 28.9 Drawing of human skin that emphasizes presence of sense organs.



Skin Receptors in Humans

Human Skin Model

1. With the help of figure 28.9 and a model of the skin if available, find these *areas* or *structures*.
2. Describe or name where each is *located*.

a. subcutaneous layer _____

b. adipose tissue _____

c. dermis _____

d. epidermis _____

e. hair follicle and hair _____

f. sebaceous gland _____

g. sweat gland _____

h. sense organs _____

Experimental Procedures

Touch

1. The subject is seated, with the *eyes closed*.
2. The experimenter tests the subject's ability to discriminate 2 points of a pair of scissors, an open large paper clip or bobby pin, or an aestheometer, in the areas listed following.

3. Hold the points of a pair of fine scissors on the given skin area, with both of the scissor points (simultaneously) *gently touching* the subject. The subject should respond as to whether the experience is one or 2 touch sensations.
4. Record the shortest distance between the scissor points for a 2-point discrimination.

   a. forearm _____

   b. back of the neck _____

   c. index finger _____

   d. back of the hand _____

Which of these areas contain the greatest density of receptors for touch? _____ How do you know?

_____

_____

Heat and Cold

1. Fill 3 1,000 ml beakers as follows: *ice water* (≈ 1° C), *tap water* at room temperature (≈ 22° C); *warm water* at 45° C.
2. Place your *left hand* in the first beaker and your *right hand* in the third beaker.
3. Keep both hands immersed for *30 seconds*.
4. Place both hands in the second beaker.
5. Record the sensation in the right and left hands.

   a. right hand _____

   b. left hand _____

Explain these results. _____

Pain

1. Make a *grid* using a ball point pen 2 cm square and blocked off into ½ cm on the volar (palm) side of the arm near the wrist, the back of the hand, and the ball of the thumb of the subject (fig. 28.10). The experimenter uses a sharp needle or pin and presses it into all areas of the divisions of the grid. Taking care *not* to *penetrate* the skin, the experimenter should include 4 spots in each of the smallest squares.
2. Record a positive response by making a dot with a pencil on the grid in figure 28.10 corresponding to the same area on the skin.
3. Determine the number of pain spots per square centimeter for each area:

   _____ volar side of arm;

   _____ back of hand;

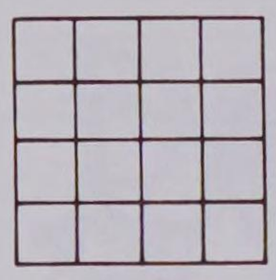
   _____ ball of thumb.

Which area contains the most receptors for pain? _____

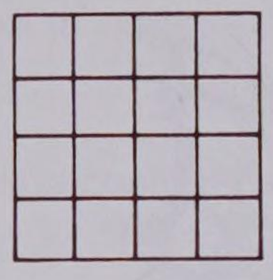
How do you know? _____

_____

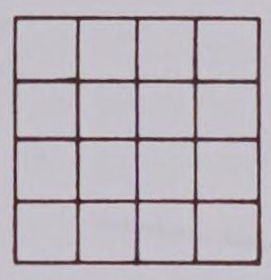
Figure 28.10 Grid drawings to record location of pain receptors on the portions of the body. ***a.*** Volar side of arm. ***b.*** Back of hand. ***c.*** Ball of thumb.



a. Volar side of arm

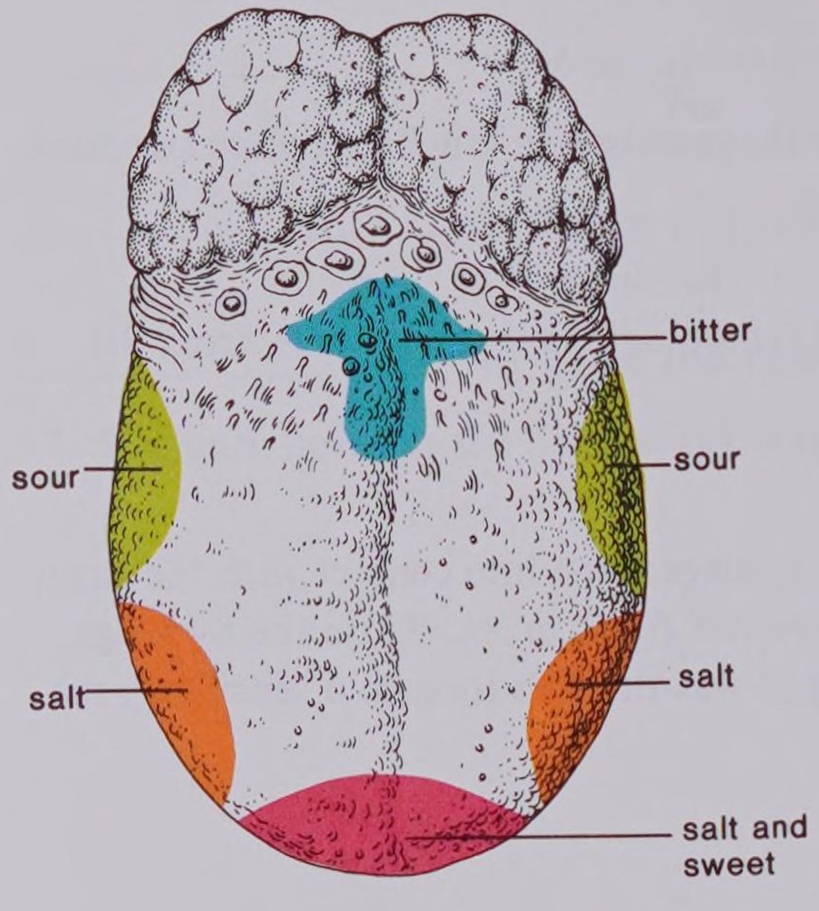


b. Back of hand



c. Ball of thumb

Figure 28.11 Human tongue. It is believed we have 4 taste sensations—sweet, sour, salty, and bitter—and that they are located on the tongue as shown.



Chemoreceptors

Taste buds and the olfactory receptors are chemoreceptors in humans (fig. 28.11). Most other animals also have receptors that respond to chemicals in the same manner. We will study only one example.

Human Response (Optional)

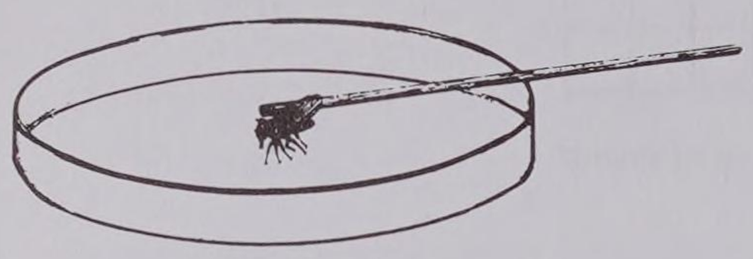
1. Wash your hands and obtain samples of 0.05M, 0.1M, 0.2M, 0.4M, 0.8M *galactose, glucose,* and *sucrose* in disposable paper cups or microbeakers. Each student should obtain his or her fresh solutions and place them in fresh cups. *Caution: Do not share container or liquids.* (Save these for the next experiment.)
2. Dip a clean finger in each solution, starting with the least and working toward the most concentrated, and *taste* until you just detect the sugar.
3. Rinse your finger between tests, using a wash bottle. (Collect the water in a beaker.) What was your *threshold sensitivity* to each sugar?

Galactose _____

Glucose _____

Sucrose _____

Figure 28.12 ***a.*** Fly attached to rod is lowered toward food until feet touch the surface. ***b.*** Proboscis retracted. ***c.*** Proboscis fully extended.



a.

b.

c.

Blowfly Response

Flies have chemoreceptors on their legs. When they're ready to feed, they lower the proboscis, a tubelike feeding structure (fig. 28.12*c*).

1. Obtain a styrofoam block that contains *sticks* with *flies* attached.
2. Obtain 3 plastic testing plates, one for each sugar, and mark the wells according to sugar solutions, from the least to the most concentrated.
3. Fill the wells with the *sugar solutions* you have just tested on yourself.
4. Fill an additional well with water.
5. Give the fly a drink of *water* before you begin. Hold the fly so that its forelegs come into contact with the sugar solutions (fig. 28.12*a*), starting with the least concentrated. *Do not allow the fly to drink.* Rinse the fly's legs, using a wash bottle, between tests. (Collect the water in a beaker). What was the fly's threshold sensitivity to each sugar?

Galactose _____

Glucose _____

Sucrose _____

Compare your sensitivity to that of the fly. _____

_____

Laboratory Review 28

_____ 1. What 2 types of sense organs are found in the ear?

_____

_____ 2. What is the function of each of the following?

_____ a. lens

_____ b. retina

_____ c. cornea

_____ d. pupil

_____ 3. What are the receptors for sight?

_____ 4. Ear infections most likely settle where?

_____ 5. When you yawn, you will sometimes notice a change in pressure in your ears. What has happened?

_____ 6. Taste and smell are what type of receptors?

7. If a burn destroys the dermis, is the sensation of pain in that area still possible? Why or why not?

References

DiPierro, J., and Ragonese, R. 1972. *Principles of Biology.* Dubuque, IA: Kendall/Hunt Publishing Company.

Snow, B. L., ed. *Biological Laboratory Exercises.* Boston, MA: Suffolk University.

Laboratory

29

Musculoskeletal System

Learning Objectives

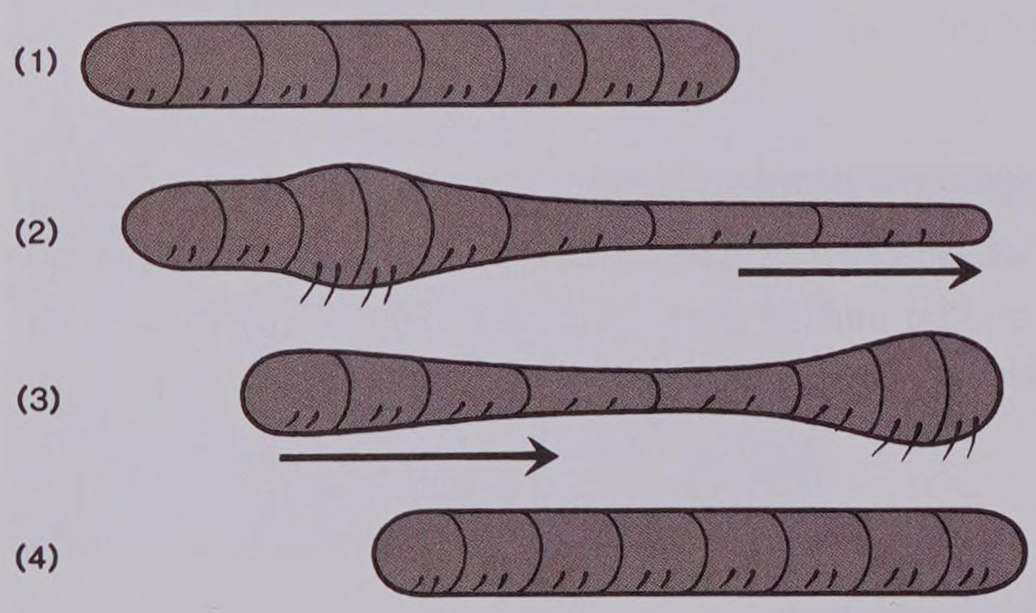
Students should be able to

1. identify and describe the microscopic structure of compact bones, hyaline cartilage, and skeletal muscle;
2. identify the bones of the human skeleton;
3. compare homologous forelimbs and hindlimbs of vertebrates;
4. upon examination, identify the action of a particular muscle;
5. describe the recording of simple muscle twitch summation and tetanus with the physiograph;
6. describe an experiment that demonstrates the contractile nature of muscle protein filaments.

Skeletons

Skeletons provide a framework, help protect internal organs, and assist movement. To cause body movements, the force of muscular contractions must be specifically directed against other parts of the body. In some animals, such as segmented worms, the pressure of muscular contraction is applied to fluid-filled body compartments (fig. 29.1); therefore, the animal is said to have a *hydrostatic skeleton.* Animals with hydrostatic skeletons are not as capable of complex movements as are animals with a rigid, supporting, exo- or endoskeleton. *Exoskeletons* are found among such diverse animals as corals, mollusks, and arthropods. The protective exoskeleton of arthropods is composed largely of chitin, which, as the animal grows, must be periodically shed by molting. The *endoskeleton* of vertebrates, which is composed of cartilage and bone, grows with the animal. Those tissues are the storage areas for calcium and phosphorus; they are also the site of the production of blood cells in adults.

Figure 29.1 Locomotion in a hypothetical 20-segment earthworm in which longitudinal muscles apply pressure against fluid-filled body segments. When longitudinal muscles contract, body portions expand, and setae (bristles) protrude into the soil. When circular muscles contract, body portions constrict, and setae are removed. This pattern allows the worm to move forward as illustrated in sketches 1–4.



Bone and Cartilage

A long bone, such as the human femur, illustrates the principles of bone anatomy. When the bone is split open, as in figure 29.2, the longitudinal section shows that it is not solid but has a cavity bounded at the sides by *compact bone* and at the ends by *spongy bone*. Beyond the spongy bone there is a thin shell of compact bone and finally, a layer of *cartilage*. Spongy bone contains numerous bony bars and plates separated by irregular spaces. These spaces are often filled with *red marrow,* a specialized tissue that produces blood cells. The cavity itself usually contains *yellow marrow,* which is a fat storage tissue.

Compact Bone

S 1. Obtain a cross section slide of compact bone and notice that it shows many series of concentric rings (fig. 29.2*b,c*).

S 2. Find in the center of each series of rings, or *lamellae,* a *Haversian canal*. Each ring contains several *lacunae,* which contain living bone cells, or *osteocytes*.

S 3. Notice extensions projecting outward from each lacuna, called *canaliculi,* by which a living cell exchanges nutrients for wastes with the blood vessels in the Haversian canal.

S 4. Notice that the lacunae are separated from one another by the *matrix* the cells laid down.

Bone Strength (Demonstration)

Bone is about 75% calcium phosphate and about 25% collagen fibers. These materials, acting together, give bone its strength and flexibility.

S 1. Obtain the femurs of chickens which have been soaked in 5% to 10% acetic acid for 24 to 48 hours. Under these conditions the calcium is removed, but the protein remains.

S 2. Compare treated with untreated bones. What is the shape of the treated bone? _____

_____

Is the treated bone more flexible or less flexible than the untreated bone? _____

Is the treated bone able to withstand more direct downward compression or less direct downward compression than the untreated bone? _____

Other chicken bones have been baked in an oven at a very high temperature for several days. What remains is calcium phosphate devoid of water and without any reinforcing protein fibers. What is the effect of this treatment on bone strength? _____

_____

Hyaline Cartilage

S Study a slide of hyaline cartilage located within the trachea (fig. 29.3). Hyaline cartilage occurs at the ends of bones, in the pieces of cartilage that connect the ribs to the sternum, and in the nose, larynx, trachea, and bronchi. The cells (*chondrocytes*) are found in 2s and 3s in spaces called lacunae. The lacunae are separated by a flexible *matrix*.

Human Skeleton

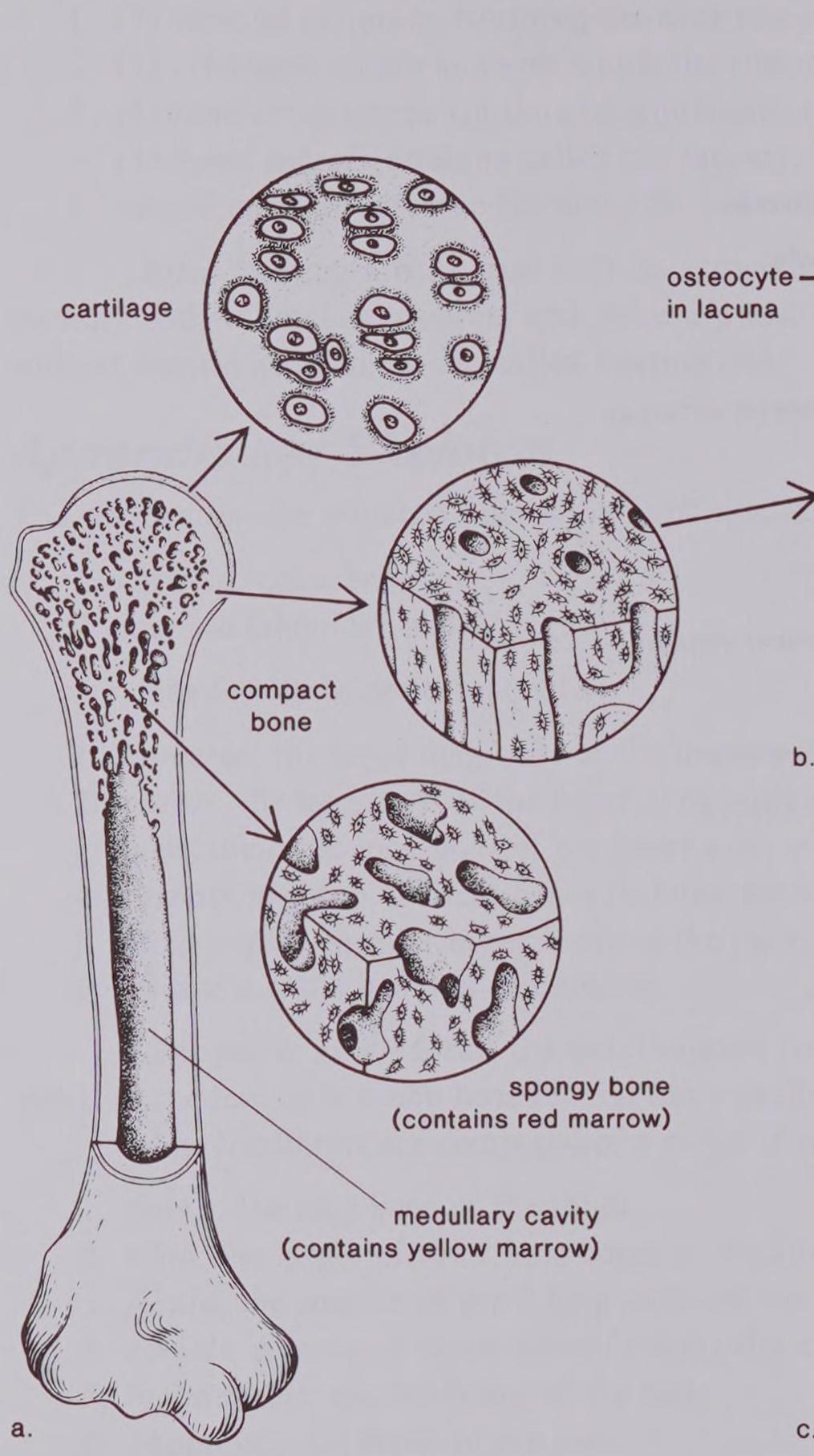
The human skeleton, like other vertebrate skeletons, is divided into axial and appendicular components (fig. 29.4) The *axial* skeleton is the main longitudinal portion. The *appendicular* skeleton includes the bones of the appendages and their supportive pectoral and pelvic (shoulder and hip) girdles.

Axial Skeleton

S The *skull* is composed of many small bones fused together. Note particularly

1. the *cranium,* a bony case enclosing the brain and major sense organs;
2. the *facial* bones;
3. the *mandible* (lower jaw), the only bone of the skull not immovably fused to the rest.

Figure 29.2 ***a.*** A long bone is encased by membrane except where covered by articular cartilage. The central shaft is composed of compact bone, but the ends are spongy bone, which can contain red marrow. A central medullary cavity contains yellow marrow. Compact bone (***b***) diagram, (***c***) Photomicrograph.



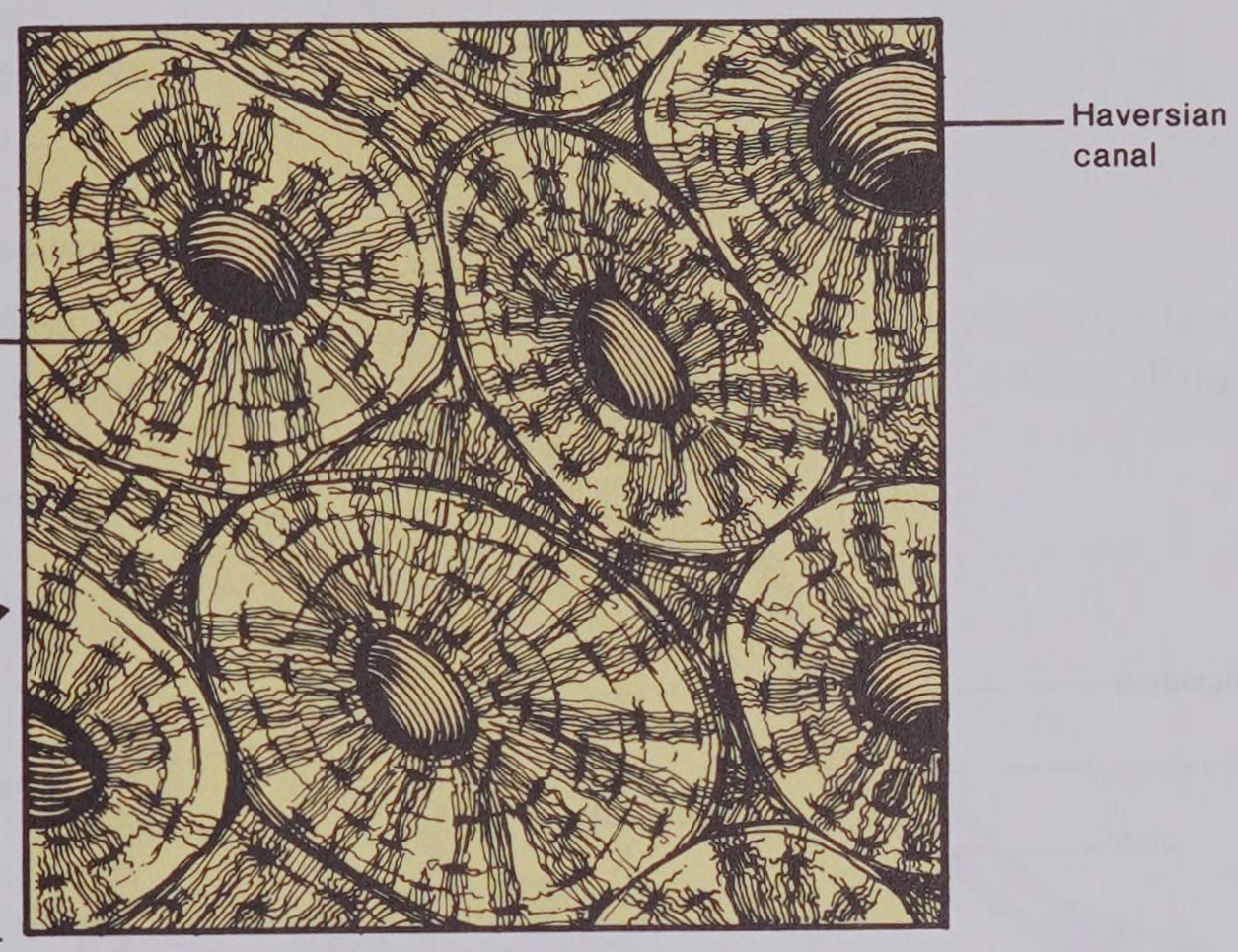
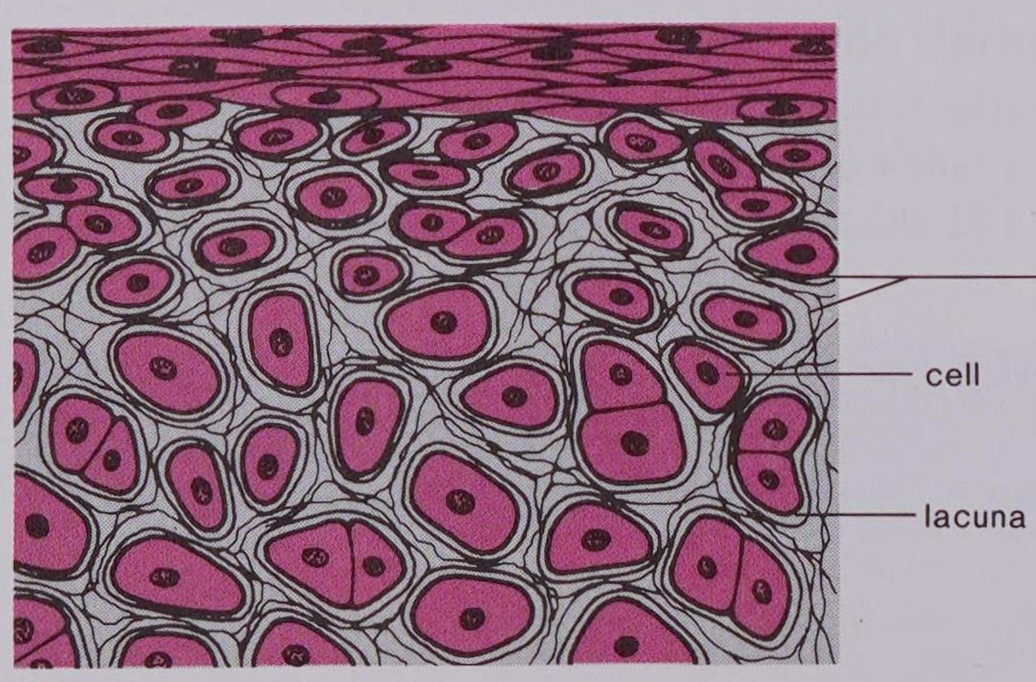


Figure 29.3 Hyaline cartilage from tracheal wall. ***a.*** Diagram of cartilage area. ***b.*** Photomicrograph.



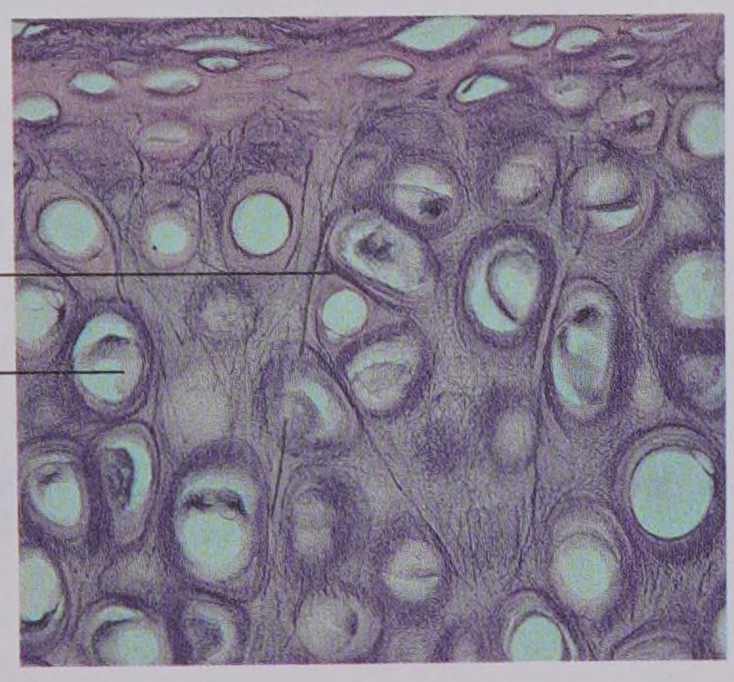
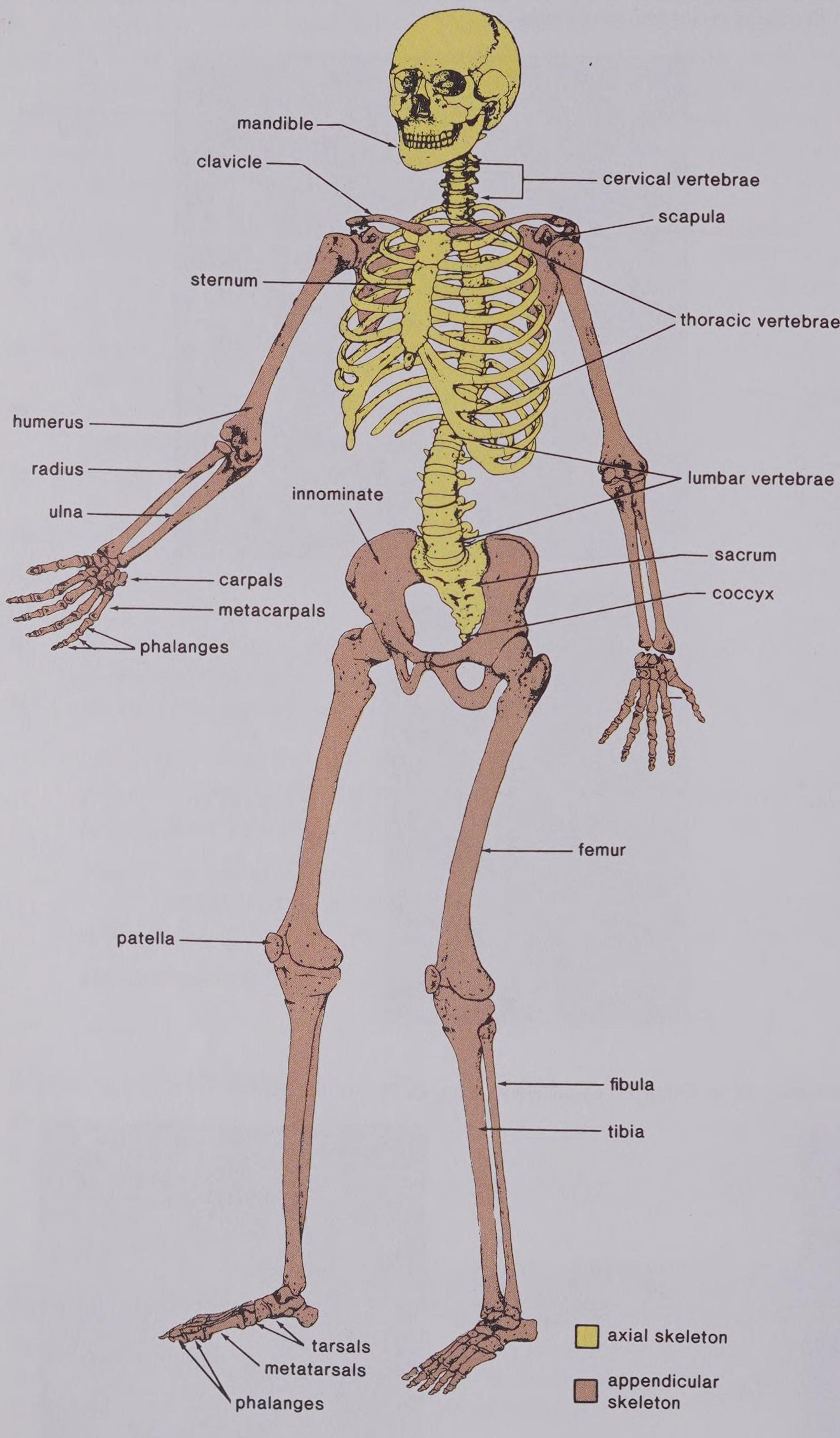


Figure 29.4 Human skeleton.



The *vertebral column* serves for support and for housing the *spinal cord*. It is composed of many vertebrae, separated from one another in life by intervertebral discs. The column is customarily divided into 5 series:

1. (7) *cervical* vertebrae (forming the neck region);
2. (12) *thoracic* vertebrae (with which the ribs articulate);
3. (5) *lumbar* vertebrae (in the abdominal region);
4. (5) fused *sacral* vertebrae called the sacrum;
5. several *caudal* vertebrae (forming the tail and fused in humans to form the coccyx).

Ribs, forming a bony case with its associated muscles, provide support for the chest wall. The ribs articulate dorsally with thoracic vertebrae, and some are also attached by cartilage directly or indirectly to the sternum. Ribs without ventral attachment are called floating ribs.

Appendicular Skeleton

The *pectoral girdle,* which supports the forelimbs, is composed of 2 bones:

1. *clavicle* (collarbone);
2. *scapula* (shoulder bone).

The forelimbs are composed of

1. *humerus,* the large long bone of the upper portion of the limb;
2. *radius,* the long bone of the lower arm, with a ball-and-socket joint at the elbow, allowing rotational motion;
3. *ulna,* the other long bone of the lower arm, with a hinge joint at the elbow, allowing motion in only one plane;
4. *carpals,* a group of small bones forming the wrist;
5. *metacarpals,* slender bones forming the palm;
6. *phalanges,* the bones of the fingers.

The *pelvic girdle* forms the basal support for the hindlimbs and is composed of the innominate (hip) bones. The pelvis in the female is much broader and more shallow, and the outlet is larger than that of the male.

The hindlimbs are composed of a series of loosely articulated bones as follows:

1. *femur,* the long bone of the thigh;
2. *tibia,* the larger of the 2 long bones of the shank;
3. *fibula,* the smaller of the 2 long bones of the shank;
4. *tarsals,* a group of small bones forming the ankle;
5. *metatarsals,* slender bones of the foot;
6. *phalanges,* the bones of the toes.

Vertebrate Skeleton Comparisons

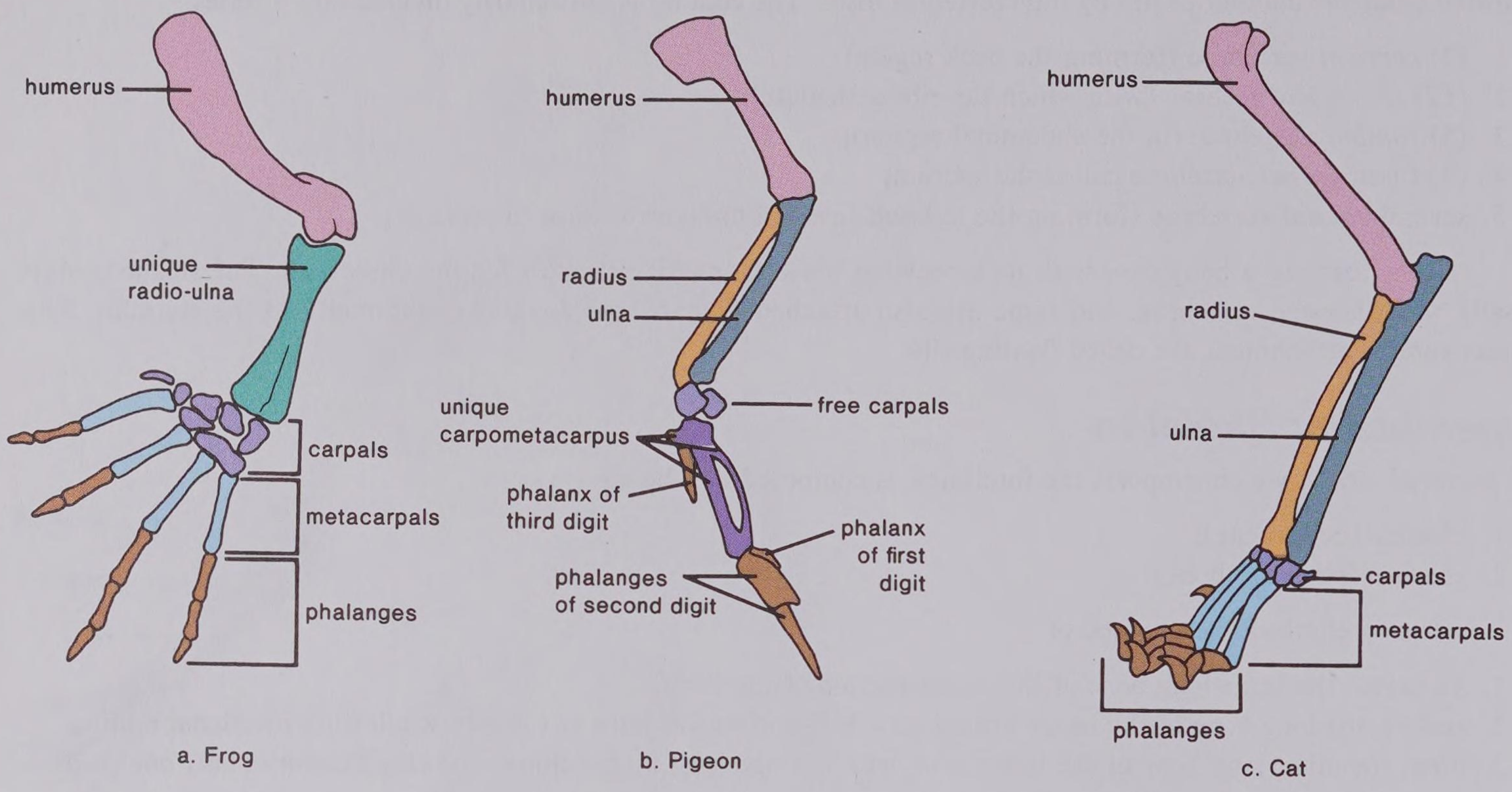
Comparison Between Forelimbs of Various Vertebrates

Vertebrates are said to have *homologous* structures, structures that have a similar construction but differ in their function. Compare, using the actual skeletons that are available and figures 29.4 and 29.5, the forelimbs of the various vertebrates that are available and show in table 29.1 that they contain *homologous parts* but differ in function.

Table 29.1 Comparison of Forelimbs (figs. 29.4 and 29.5)

Animal	Bones	Functions
Frog		
Bird		
Cat		
Human		

Figure 29.5 Forelimbs of (***a***) frog, (***b***) bird, and (***c***) cat.



Comparison Between an Animal's Forelimbs and Hindlimbs

S 1. Considering these same animals, using the actual skeletons that are available and figures 29.4 and 29.6, compare the forelimb with the hindlimb of the same animal.

S 2. If a dramatic difference is apparent, explain this difference (in table 29.2) on the basis of the function of each. What portion of the foot touches the ground and is used for support in the frog, bird, cat, and human?

frog _____ ; bird _____ ; cat _____ ;

human _____ .

Does this difference seem to be an adaptation to the function of the foot in each animal? _____

S 3. Compare the skeleton of a fish to that of a snake. _____

_____

_____

Can you correlate the reduced girdles in fish and the absence of girdles in snakes to their means of locomotion?

_____

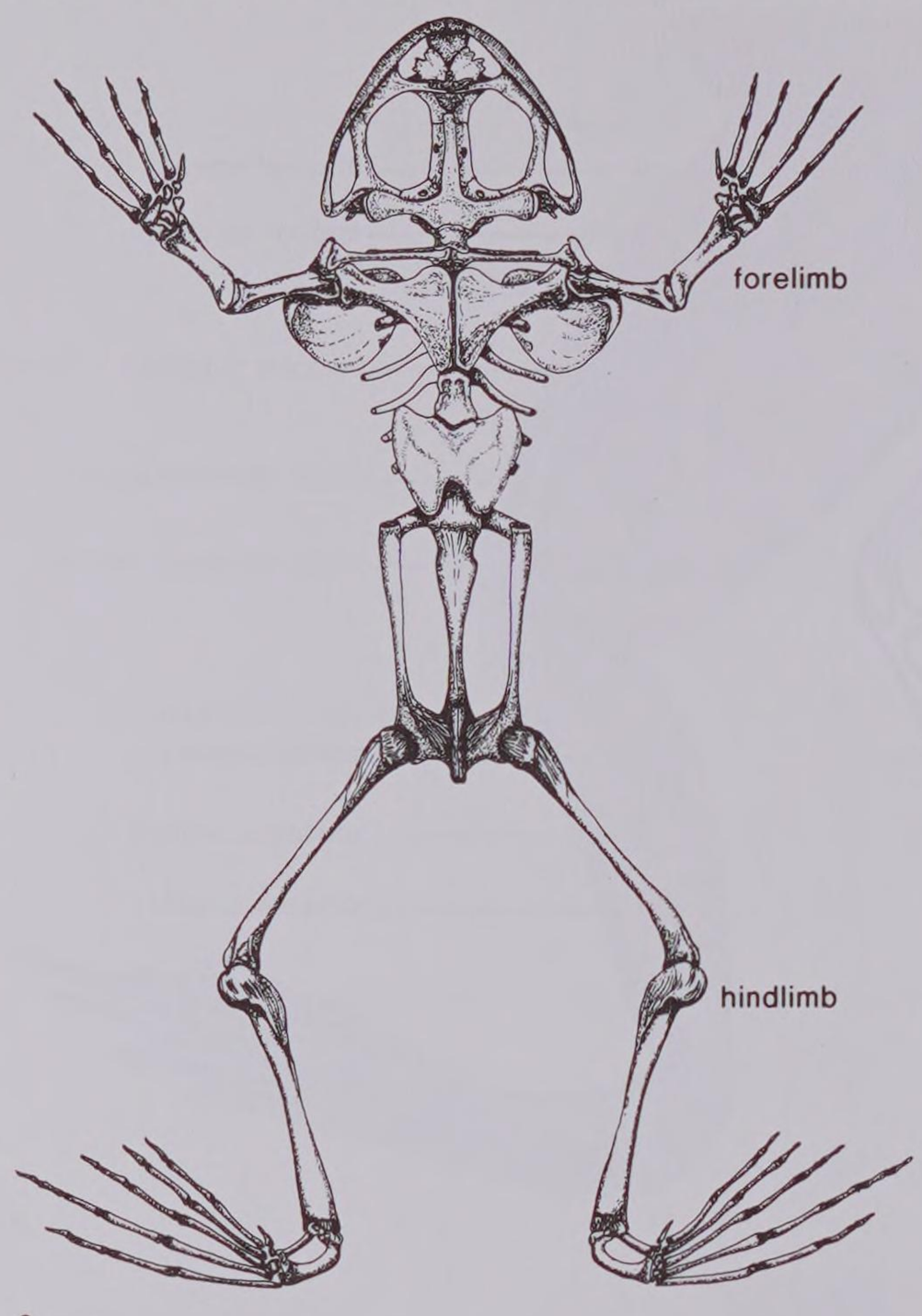
_____

Your instructor may have additional comments about the skeletons on display.

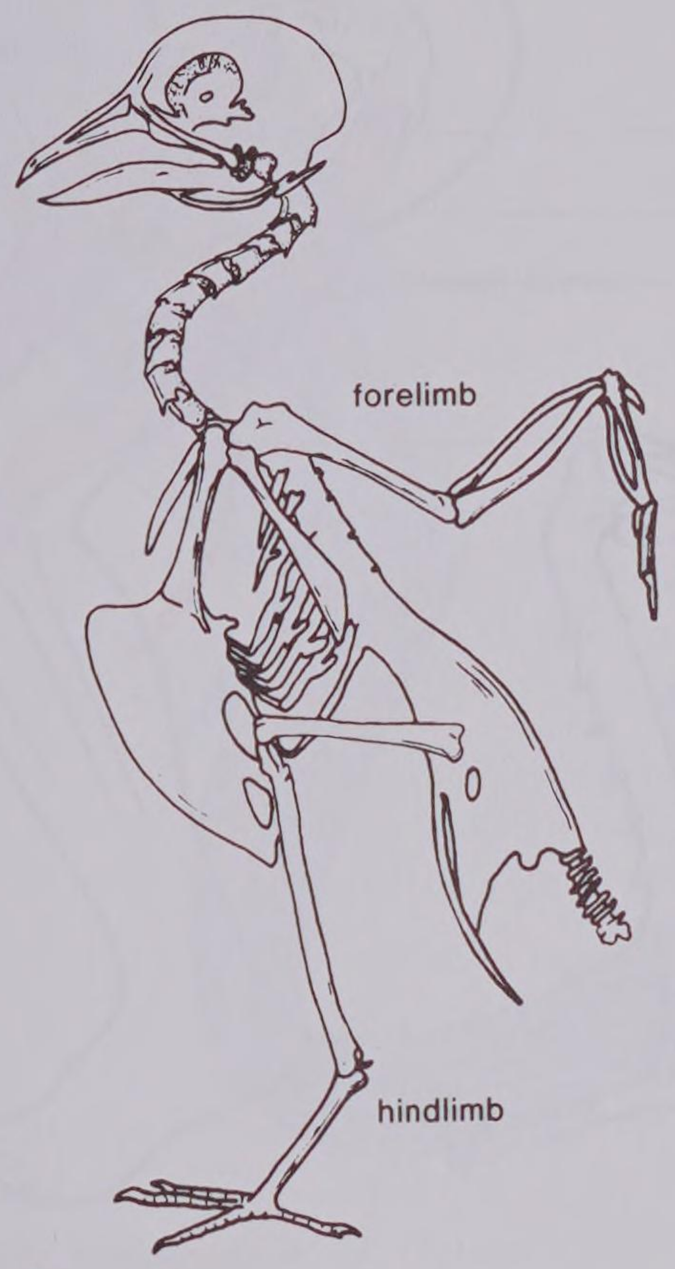
Table 29.2 Comparison of Forelimb and Hindlimb (figs. 29.4 and 29.6)

Animal	Forelimb	Hindlimb	Explanation
Frog			
Bird			
Human			
Cat			

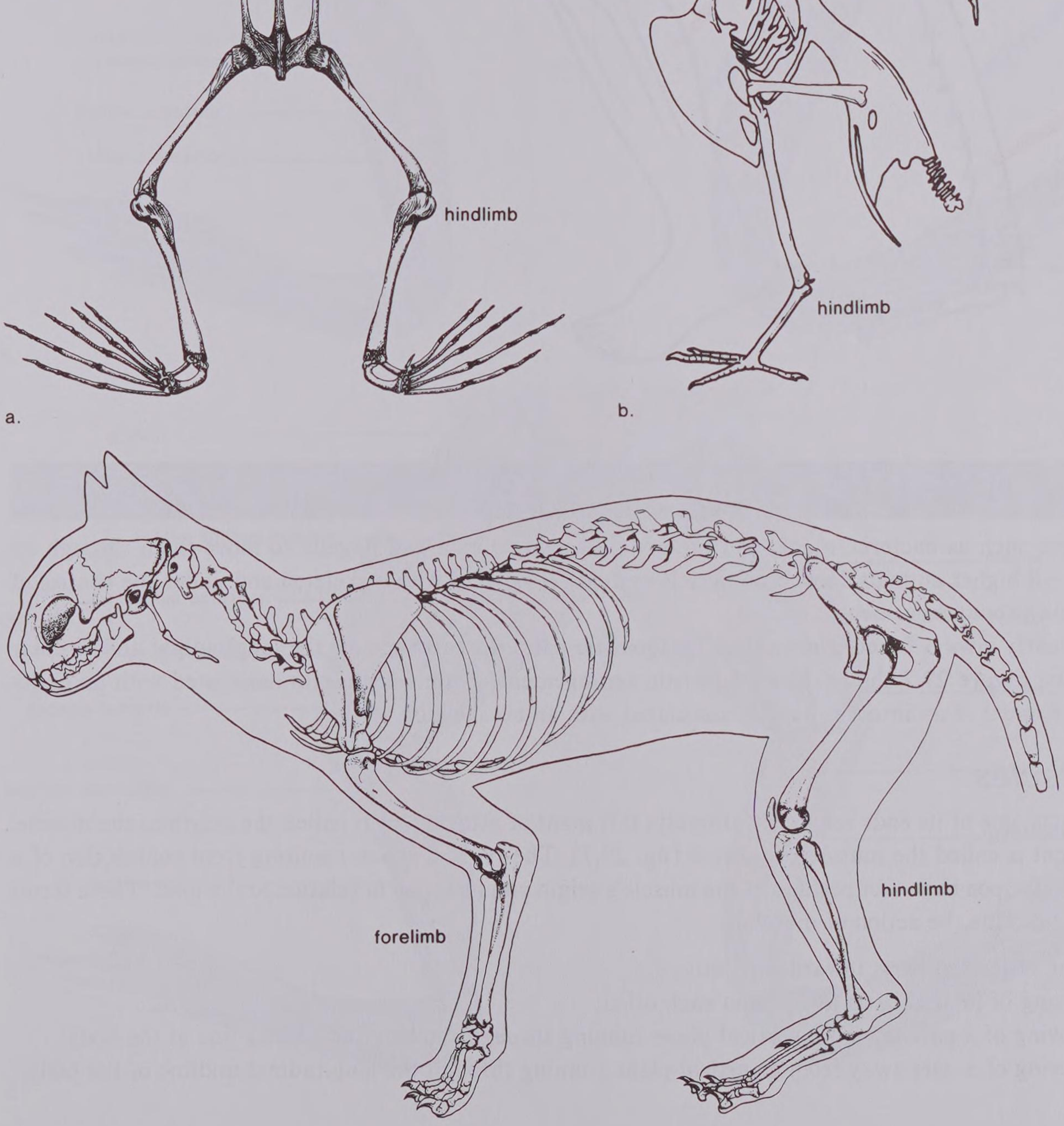
Figure 29.6 Animal skeletons. ***a.*** Frog. ***b.*** Pigeon. ***c.*** Cat.



a.

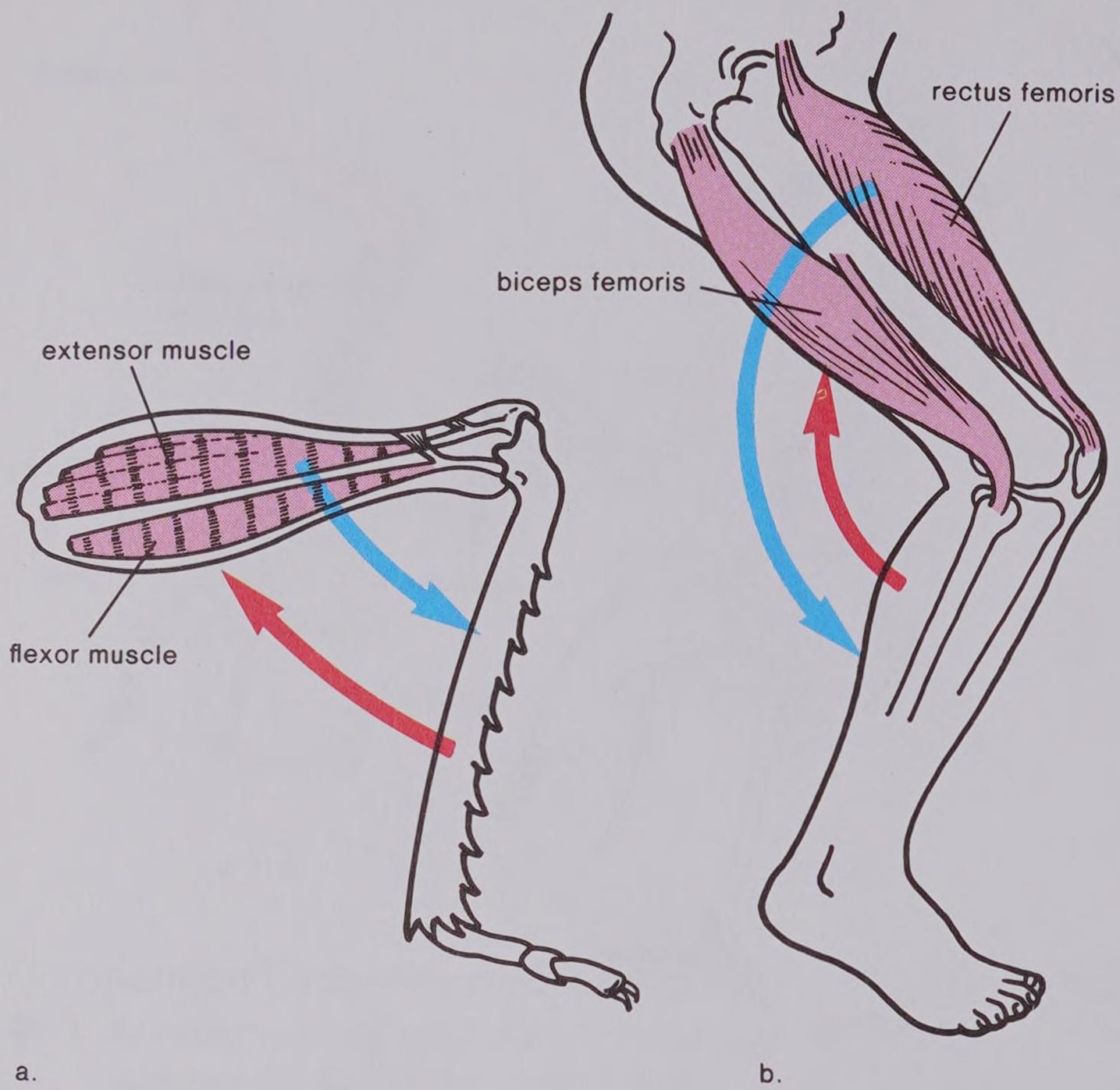


b.



c.

Figure 29.7 Antagonistic pairs of muscles in (***a***) insect limb and (***b***) human leg. Arrows indicate direction of movement of lower limb when muscles in upper limb contract.



Muscles

Simple small organisms, such as bacteria, algae, and protozoa, depend on cilia and flagella to move them through an aqueous environment. All higher animals depend on muscle systems associated with a skeleton and under the control of a nervous system to bring about movement.

Muscles are nearly always found arranged as antagonistic pairs. In earthworms, the longitudinal and circular muscles are antagonists. Figure 29.7 shows the antagonistic arrangement of human muscles associated with an endoskeleton and the arrangement of an insect's muscles associated with an exoskeleton.

Actions of Muscles

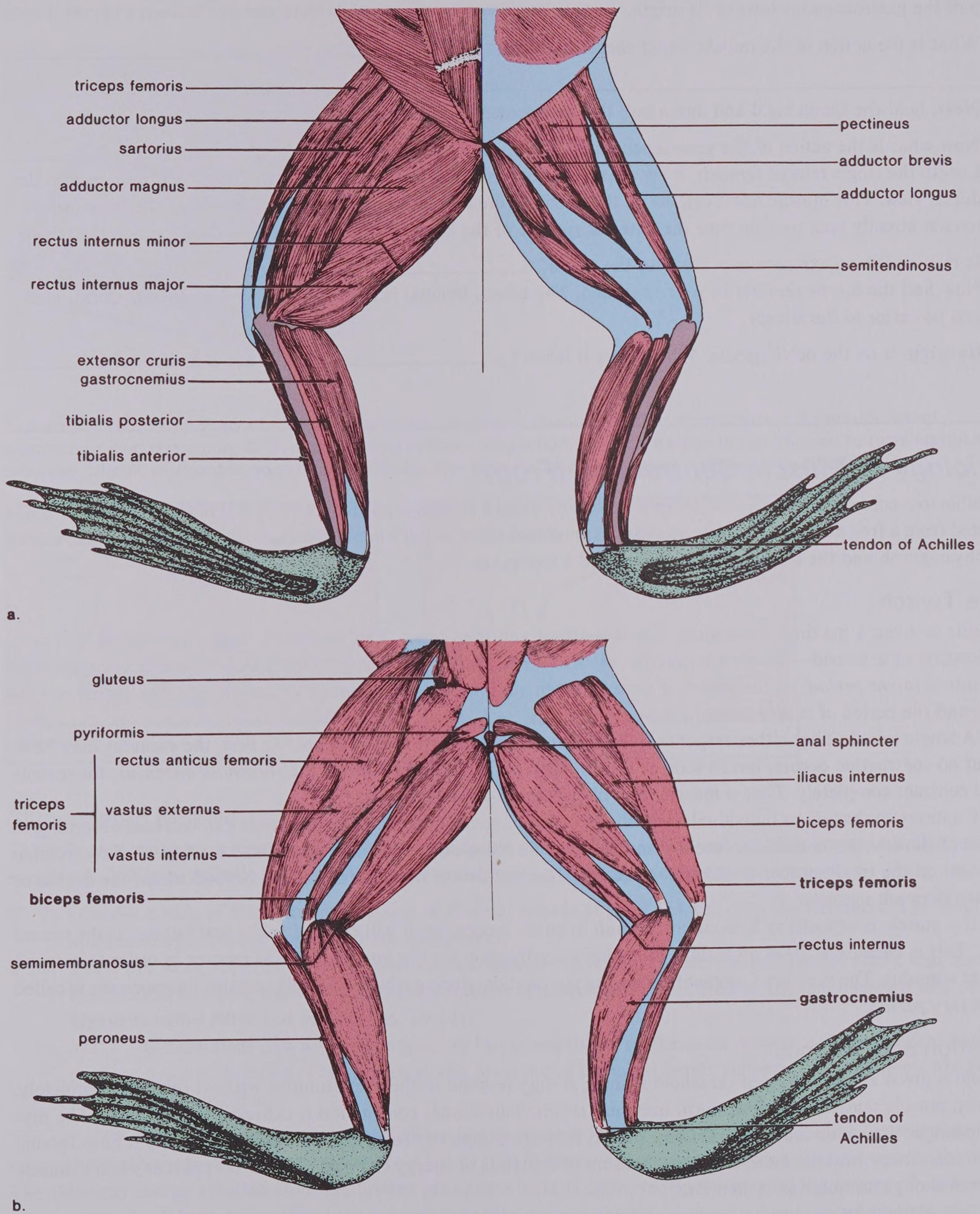
When a muscle contracts, one of its ends remains stationary; this point of attachment is called the *origin* of the muscle. The movable attachment is called the muscle's *insertion* (fig. 29.7). The type of action resulting from contraction of a particular muscle depends upon the exact position of the muscle's origin and insertion in relation to the joint. These terms are commonly used to describe the action of muscles:

1. *flexion*—moving of jointed parts toward each other;
2. *extension*—moving of jointed parts away from each other;
3. *adduction*—moving of a part toward a vertical plane running through the longitudinal midline of the body;
4. *abduction*—moving of a part away from a vertical plane running through the longitudinal midline of the body.

Frog Dissection

S 1. Study the arrangement of muscles in the hindleg of a preserved frog, using figure 29.8 as a guide.
S 2. Locate the *gastrocnemius,* a large muscle on the back of the shank.
S 3. Observe the white connective tissue (fascia) surrounding the fleshy belly of the muscle. The fascia continues beyond the muscle at either end to form strong white bands, the *tendons,* that attach the muscle to bone.

Figure 29.8 Frog, muscles of hindlegs. ***a.*** Ventral view. ***b.*** Dorsal view.



4. Hold the thigh of the frog rigid with one hand and grasp the belly of the gastrocnemius with the other. Gently pull the gastrocnemius toward its origin.

   What is the action of the muscle under these conditions? _____

   _____

5. Next, hold the shank rigid and again pull the gastrocnemius toward its origin.

   Now what is the action of the gastrocnemius? _____

6. Locate the frog's *triceps femoris,* a very large muscle on the anterior surface of the thigh. It is best seen in the dorsal view. This muscle has 2 origins on the pelvic girdle and inserts on the femur by way of the broad white tendon already seen passing over the anterior surface of the knee. Hold the thigh firmly and pull on the triceps.

   Is this muscle an extensor or a flexor of the shank? _____

7. Now find the *biceps femoris* in your specimen. The biceps femoris is a narrow muscle best seen in dorsal view just posterior to the triceps.

   Its origin is on the pelvic girdle. Where does it insert? _____ What is its action? _____

   _____

Physiology of Muscle Contraction (Frog)

It is possible to record the contraction of a whole muscle by using a physiograph (fig. 29.9). A calf muscle (*gastrocnemius*) is dissected from a frog and mounted. The muscle is stimulated electrically; when it contracts, its movements are recorded by the physiograph and the resulting pattern is called a myogram.

Muscle Twitch

If a muscle is given a maximum stimulus, it will contract and then relax. This action—a single contraction that lasts only a fraction of a second—is called a muscle *twitch.* Figure 29.10*a* is a myogram of a twitch, which is customarily divided into a *latent period,* or the period of time between stimulation and initiation of contraction; the period of *contraction;* and the period of *relaxation.*

A single muscle fiber either responds to a stimulus and contracts or it does not. At first, the stimulus may be so weak that no contraction occurs, but as soon as the strength of the stimulus reaches the *threshold stimulus,* the muscle fiber will contract completely. Thus a muscle fiber obeys the *all-or-none law.*

Contrary to that of an individual fiber, the strength of contraction of a whole muscle can increase according to the degree of stimulus beyond the threshold stimulus. A whole muscle contains many fibers, and the degree of contraction is dependent on the total number of fibers contracting. The *maximum stimulus* is the one beyond which the degree of contraction does not increase.

If a muscle is exposed to 2 maximum stimuli in quick succession, it will respond to the first but not to the second stimulus. This is because it takes an instant following a contraction for the muscle fibers to recover in order to respond to the next stimulus. The very brief moment, following stimulation, during which a muscle remains unresponsive is called the *refractory period.*

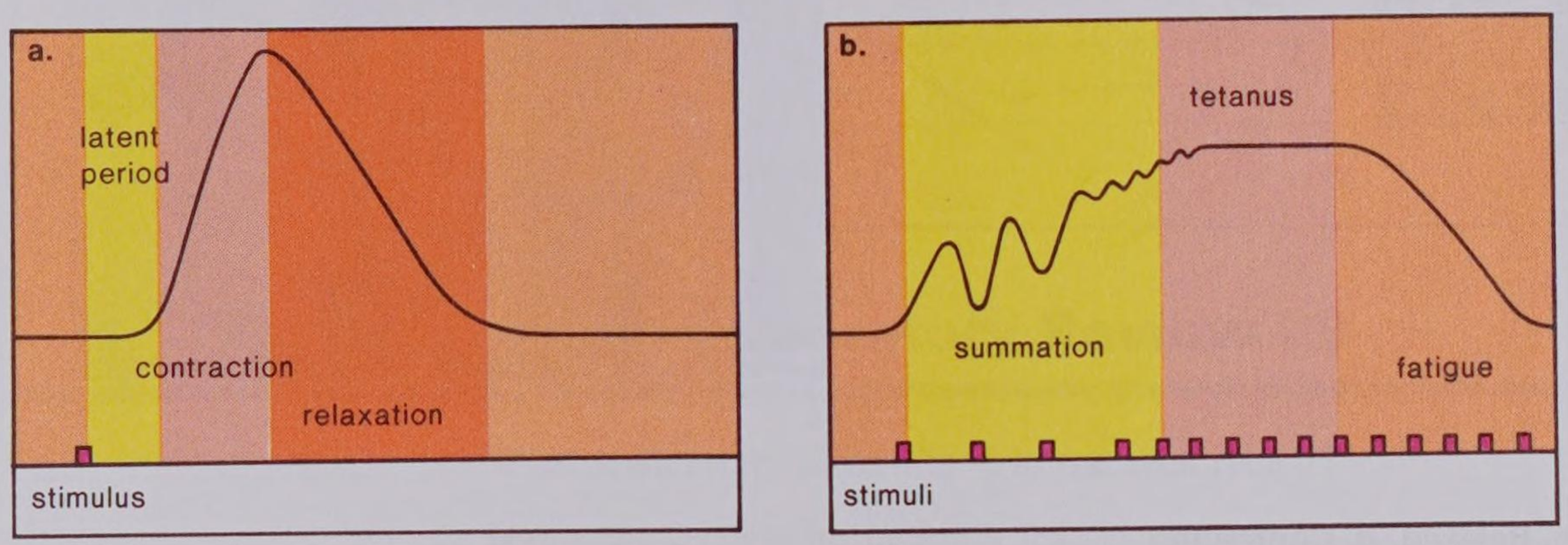
Summation and Tetanus

If a muscle is given a rapid series of threshold stimuli, it may respond to the next stimulus without relaxing completely. In this way, muscle tension *summates* until maximal *tetanic* (sustained) *contraction* is achieved (fig. 29.10*b*). The myograms no longer show individual contractions; rather, they are completely fused and blended into a straight line. Tetanic contraction continues until the muscle fatigues because of depletion of energy reserves. *Fatigue* is apparent when a muscle relaxes even though stimulation is continued.

Tetanic contractions occur whenever skeletal muscles are being actively used. Ordinarily, however, only a portion of any particular muscle is involved—while some fibers are contracting, others are relaxing. Because of this, intact muscles rarely fatigue completely.

Figure 29.9 A physiograph. This apparatus can be used to record a myogram, a visual representation of the contraction of a muscle that has been dissected from an animal.

Figure 29.10 Physiology of muscle contraction. ***a.*** Simple muscle twitch is composed of 3 periods: latent, contraction, and relaxation. ***b.*** Summation and tetanic contraction. When a muscle is not allowed to relax completely between stimuli, the contractions increase in size and then the muscle remains contracted until it fatigues.



Glycerinated Muscle

The cells that make up a whole skeletal muscle are called *muscle fibers*. Each cell contains contractile elements called *myofibrils*. When myofibrils are sectioned, *striations* (bands of light and dark regions) are seen.

Ⓢ 1. Observe a slide of a longitudinal section of skeletal muscle (fig. 29.11) and note the striations. The longitudinal units running across the myofibril are *sarcomeres*.

Ⓢ 2. These are more apparent with the electron microscope (fig. 29.11*c*). Electron microscopy study, together with biochemical analysis, has shown that the striations of a myofibril are caused by the arrangement of 2 protein filaments called *actin* and *myosin* (fig. 29.12).

Contractions of a myofibril appear to be accomplished by the actin filaments sliding toward each other, past the myosin filaments. The formation and breaking of chemical bonds between the 2 filaments are believed to cause this movement.

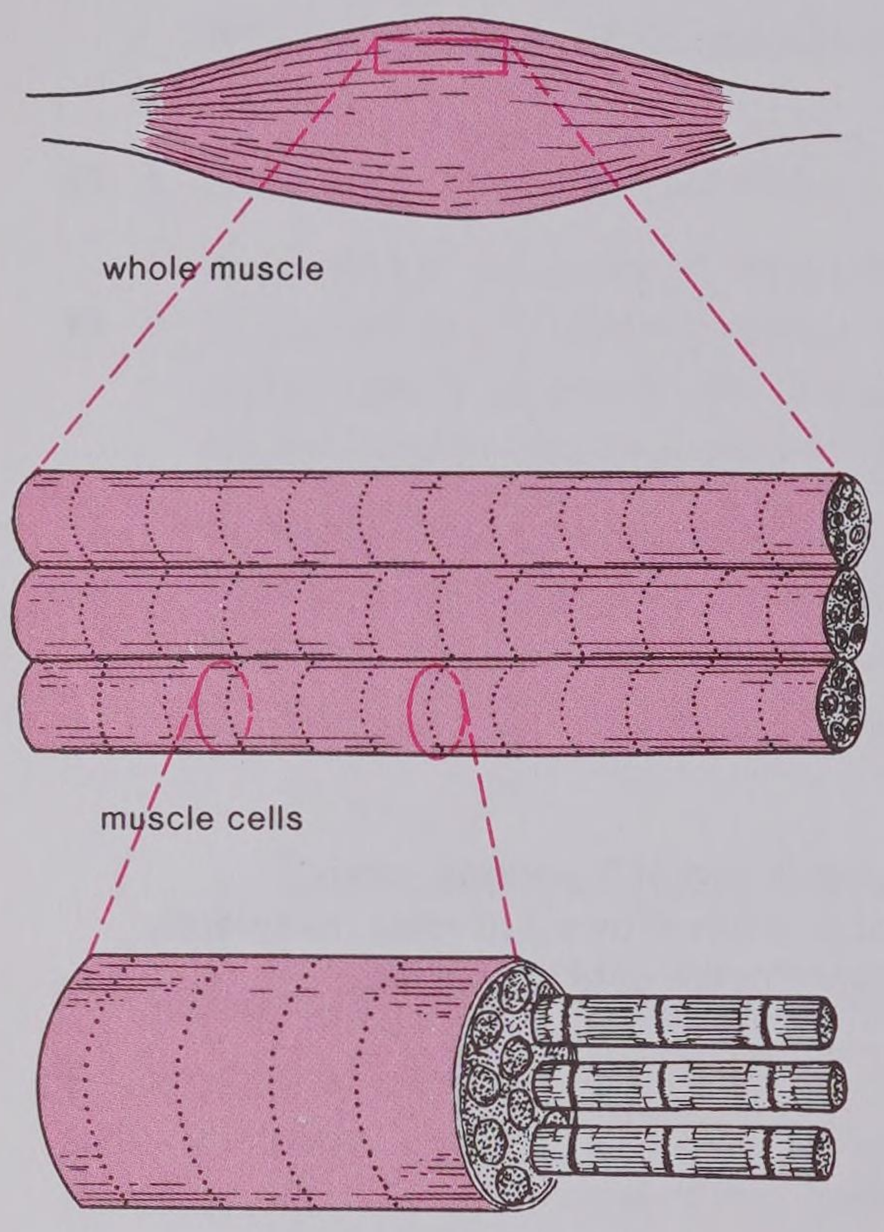
ATP serves universally as the immediate energy source for all cell reactions including contraction. When a muscle fiber contracts, ATP is broken down to ADP + P. It is believed that myosin serves as the enzyme for this reaction. The chemical energy released from the broken phosphate bond is converted to the mechanical energy of contraction.

It is possible to demonstrate the requirements for muscle contraction by this exercise, which uses glycerinated muscle. The muscle was removed either from a rabbit or a frog and was placed in a solution of 50% glycerol.

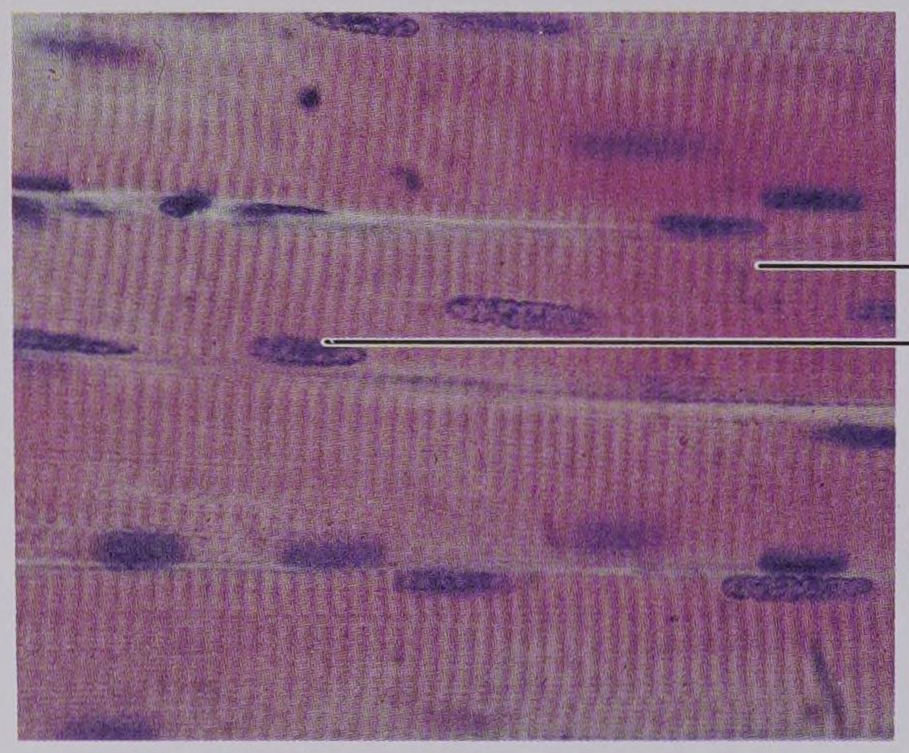
Ⓢ 1. Obtain a muscle that has been separated into very thin groups of fibers.

Ⓢ 2. Mount one of the strands in a drop of *glycerol* on a slide. Place the slide on a millimeter ruler and measure the length of the strand.

Figure 29.11 A whole muscle is made up of muscle fibers (***a***) within the muscle fibers are myofibrils. ***b.*** Photomicrograph of longitudinal section of muscle fibers. ***c.*** Electron micrograph of longitudinal section of myofibrils.



a.



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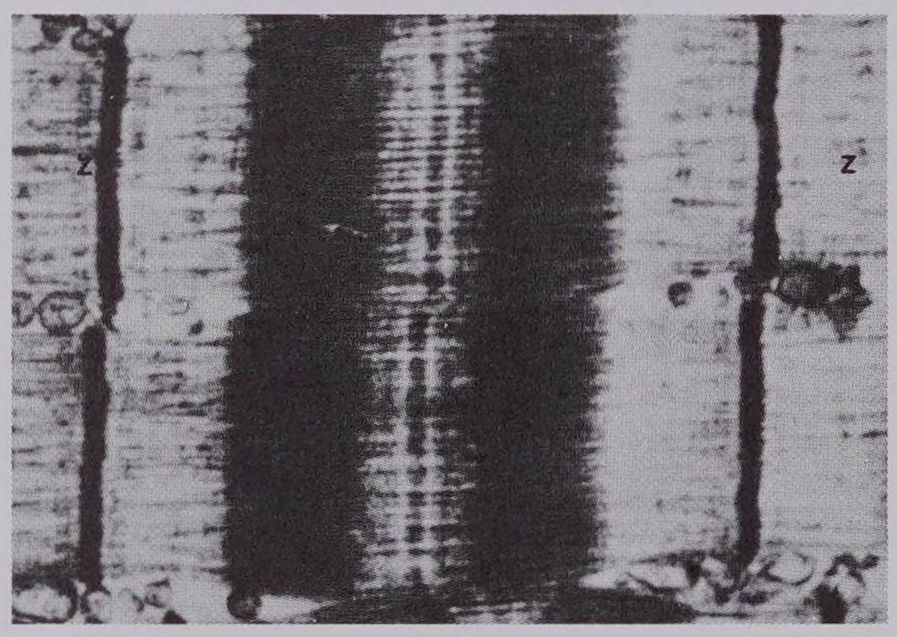
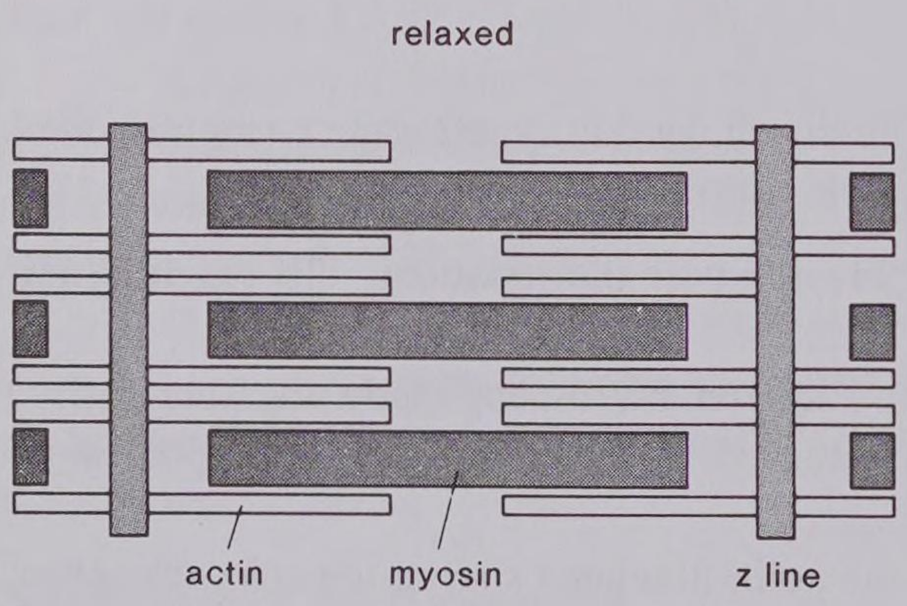
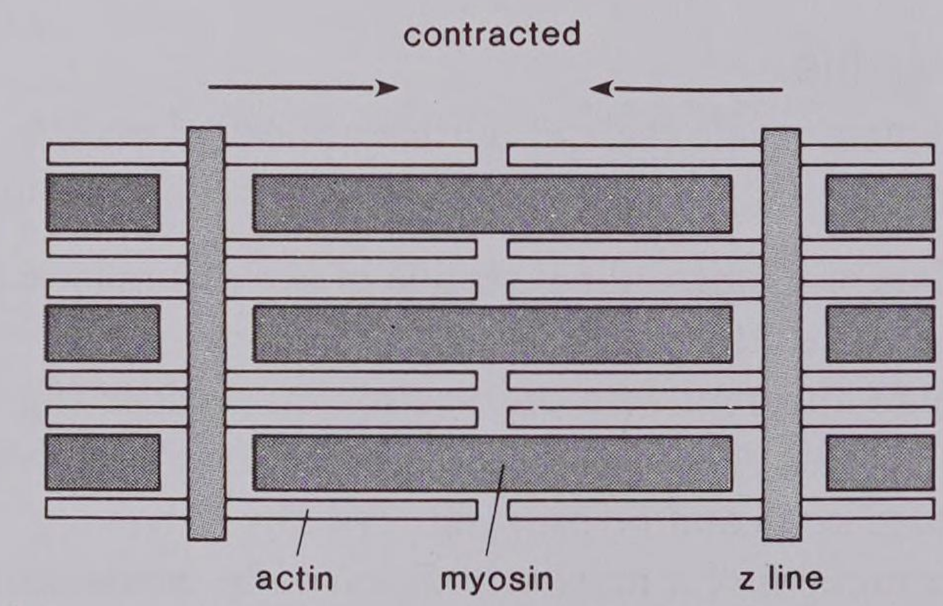


c.

Figure 29.12 Sarcomere. ***a.*** Relaxed. ***b.*** Contracted.



a.



b.

3. Record this length in table 29.3.
4. If there is more than a small drop of glycerol on the slide, soak up the excess on a piece of lens paper held at the edge of the glycerol farthest from the fibers.
5. Now add the solutions listed in the table in either order, but measure the change in length after each addition. A few drops are sufficient.

Table 29.3	
Solution	**Length in mm**
Original glycerol	
Ion solution	
ATP solution	

You probably observed that both the ion solution and the ATP solution are required for contraction.

Ⓢ To show that you understand the requirements for contraction, state the function of each of the substances in table 29.4.

Table 29.4	
Substance	**Function**
Myosin	
Actin	
Ions	
ATP	

Laboratory Review 29

_____ 1. What term refers to vertebrae in the neck region?

_____ 2. Name the 2 bones in the lower leg.

_____

_____ Name the 2 bones in the lower arm.

_____

_____ 3. Name the canals found in compact bone.

_____ 4. What term refers to the movable point of attachment of a muscle?

_____ 5. What is the specialized function of muscles?

_____ 6. What is the energy molecule that is necessary for muscle contraction?

_____ 7. What determines the degree of contraction within a muscle?

_____ 8. Is bone a living tissue? How would you justify your answer?

9. Arrange the following structures in order of increasing size: fibers, actin filaments, myosin filaments, muscle, myofibrils.

(1) _____ (2) _____

(3) _____ (4) _____

(5) _____

10. Explain the all-or-none law as it applies to muscle fibers.

Laboratory

30

Animal Development

Learning Objectives

Students should be able to

1. identify and describe the morula, blastula, gastrula, and neurula for the frog;
2. describe the development of the neural tube in the frog;
3. name and contrast the function of the extraembryonic membranes in humans and the chick;
4. compare the development of humans to that of the other animals studied.

Introduction

The early development of animals is quite similar. The fertilized egg or zygote undergoes successive cleavage divisions, forming a mulberry-shaped solid ball of cells called a *morula* and then a hollow ball of cells called a *blastula*. The fluid-filled cavity of the blastula is the *blastocoel*. Later, some of the surface cells fold inward, or invaginate, eventually forming a double-walled structure called a *gastrula*. The outer layer of the gastrula is called the *ectoderm;* the inner layer is the *endoderm*. Between these layers, a middle layer or *mesoderm* will arise. These 3 *germ layers* will give rise to different tissues during further development; the ectoderm will form the skin and nervous system; the endoderm will form the lining of the digestive and respiratory systems; and the mesoderm will give rise to the circulatory, muscular, and skeletal systems and to connective tissue.

Since the early stages of development are represented in the frog (fig. 30.1), we will use this animal as representative of all animals, including humans.

Frog Development—Study of Cross Sections

Cleavage

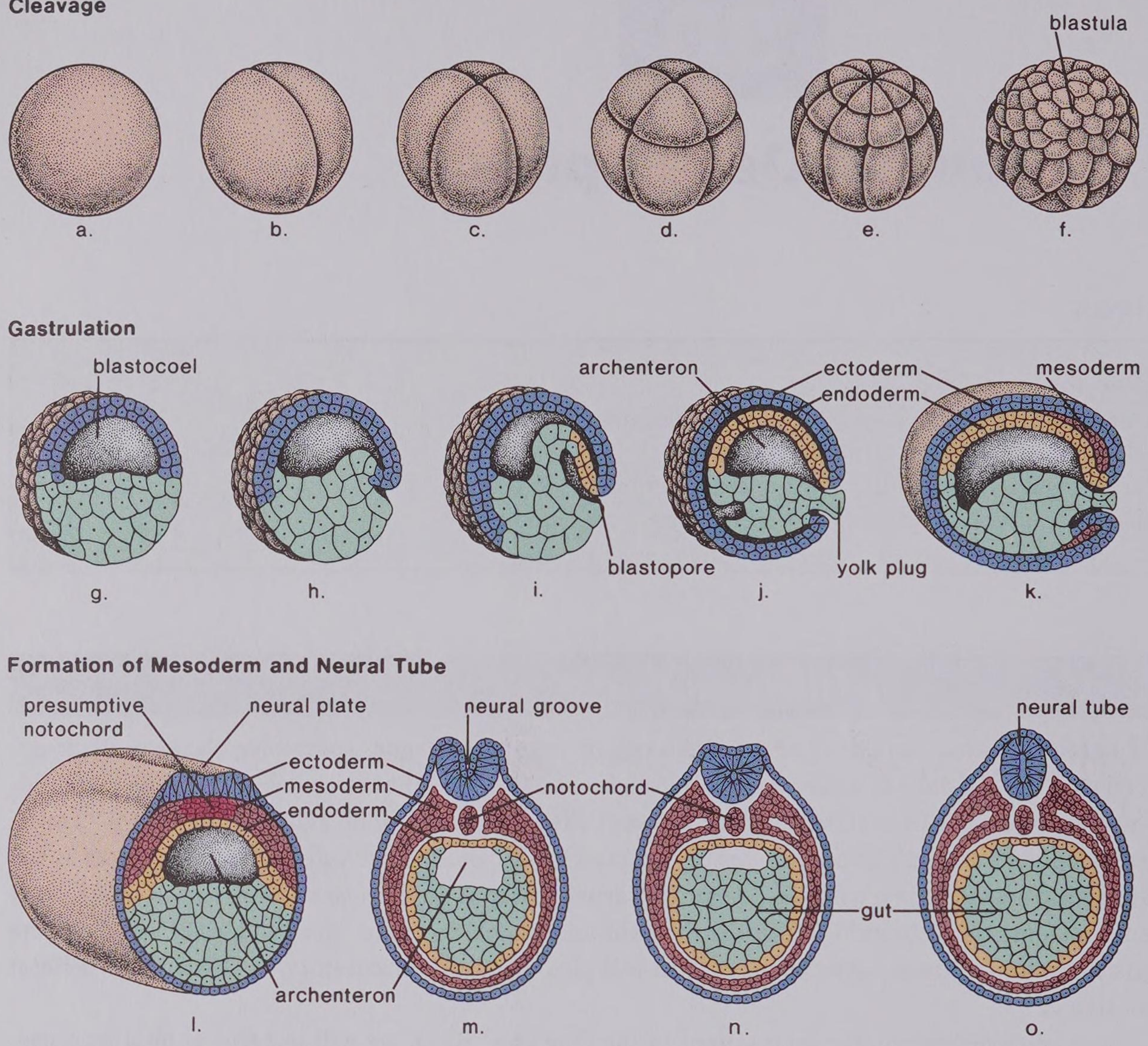
Successive division of the zygote (fertilized egg) results in 2-cell, 4-cell, 8-cell, and 16-cell stages and finally a many-celled stage called the *morula* (fig. 30.1). The morula is about the same size as the original zygote, indicating that no cytoplasmic growth has occurred yet.

S 1. Examine a slide that shows a cross section of late cleavage in the frog (or lancelet). This is the morula stage.

S 2. Draw the morula stage.

How many cells are visible in your morula? _____

Figure 30.1 Stages in the development of the frog. ***e.*** Morula. ***f.*** Blastula. ***k.*** Gastrula. ***l.*** Neurula.



Blastula

Cell division eventually results in a large number of cells that arrange themselves into a single-layered ball with a fluid-filled cavity called the *blastocoel* in the middle. This is the *blastula*. It may be possible for you to observe that the cells at the animal (upper) pole are smaller, while the cells at the vegetal (lower) pole are larger.

- S 1. Examine a slide that shows a cross section of a blastula.
- S 2. Draw this stage in the space provided.
- S 3. Label, with the aid of figure 30.1, the *animal pole* and *vegetal pole*.

Gastrula

Soon after the development of the blastula, its larger cells fold inward in such a manner that a 2-layered *gastrula* is formed. The cavity produced by the infolded layer of cells is the *archenteron,* or primitive gut, which has an opening to the outside called the *blastopore.* The outer layer of cells is the *ectoderm,* and the inner layer is the *endoderm.* As development continues, a third layer, the *mesoderm,* develops between the ectoderm and endoderm when 3 pouches form by outpocketing from the endoderm surrounding the gut. The center pouch becomes the *notochord,* a flexible skeletal rod. Just prior to the mesodermal formation of the notochord, the ectoderm produces the *neural plate,* later to become the central nervous system.

1. Examine a slide that shows a cross section of a gastrula.
2. Draw this stage in the space provided.
3. Label, with the aid of figure 30.1, all of these structures: *archenteron, blastopore, ectoderm, endoderm, mesoderm, notochord,* and *neural plate.*

Neurula

The *neurula* is characterized by a fully formed *neural tube.* This tube arises when the edges of the neural plate become elevated and fuse. Just beneath the tube is the notochord, and to the sides of the notochord are masses of cells called *somites;* the notochord and somites are of mesodermal origin.

1. Examine a slide that shows a cross section of a late neural tube (neurula).
2. Draw this stage in the space provided.
3. Label, with the aid of figure 30.1, all of these structures: *archenteron* (primitive gut surrounded by endoderm), *notochord,* and *neural tube.*

Study of Preserved Frog Eggs

1. Obtain a small vial of preserved frog's eggs at various stages of development (fig. 30.2).
2. Pour the eggs out into a petri dish and find these stages.

Unfertilized Egg

Notice that the eggs are partially pigmented; the black side contains very little yolk (nutrient material) and is called the *animal pole;* the unpigmented yolky side is called the *vegetal pole.* This unequal distribution of yolk causes some important differences in cleavage and gastrulation.

1. Sketch this stage in the space provided at the end of this section.
2. Write the name of the stage below each sketch.
3. Shade in the pigmented part of the egg.
4. Label the *animal pole* and *vegetal pole.*

Cleavage

Cleavage begins at the animal pole. The first 2 cell divisions are in the vertical plane. The third cleavage is horizontal (fig. 30.1).

1. Sketch this stage in the space provided at the end of this section.
2. Write the name of the stage below the sketch.
3. Label the *animal pole.*

Blastula

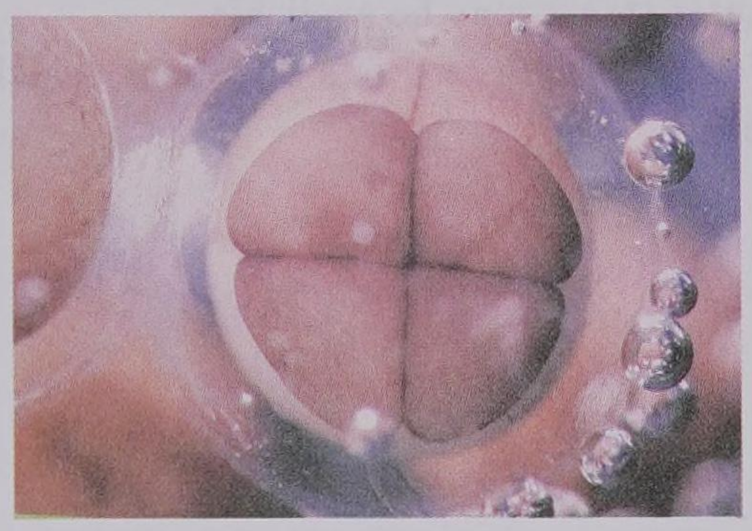
Cleavage terminates with the formation of the blastula, a ball of cells with the blastocoel in the animal half only. Notice that the cells at the animal pole are smaller than those at the vegetal pole. The latter are laden with yolk and do not participate in the formation of the blastocoel.

1. Sketch this stage in the space provided at the end of this section.
2. Write the name of the stage below the sketch.
3. Label the *animal pole.*

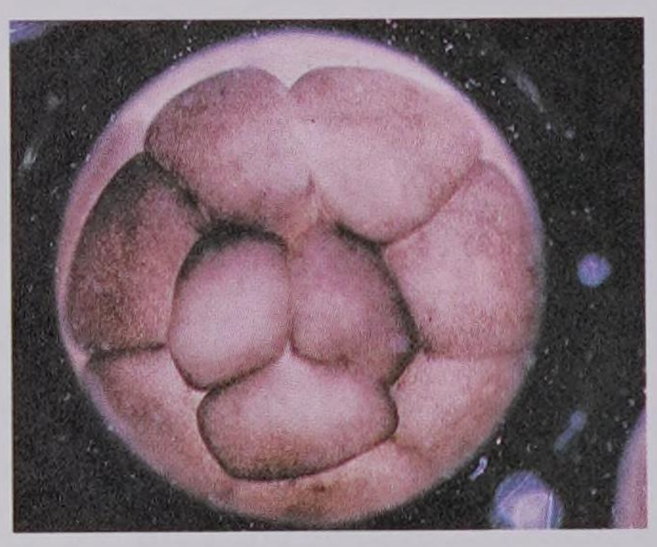
Figure 30.2 Photographs of *Xenopus* frog development. ***a.*** Fertilized egg. ***b.*** Cleavage (2-celled). ***c.*** Cleavage (4-celled). ***d.*** Morula. ***e.*** Blastula. ***f.*** Gastrula. ***g.*** Neural plate. ***h.*** Neural groove. ***i.*** Tail bud.

a.

b.

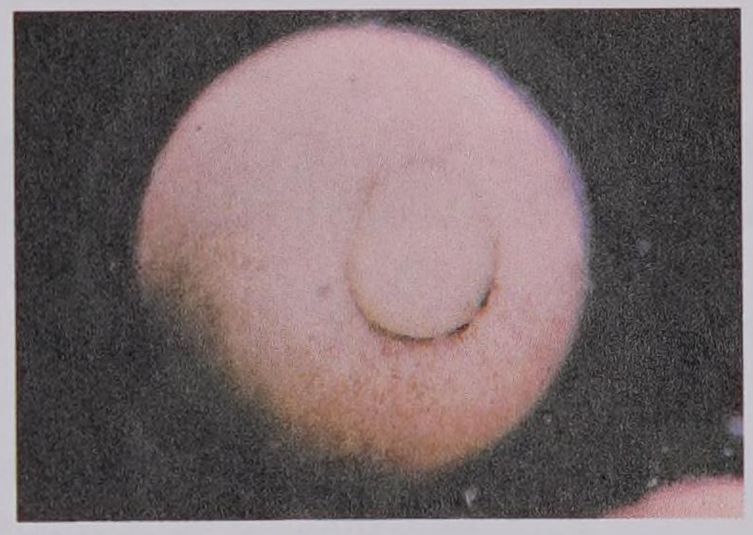


c.



d.

e.



f.

g.

h.

i.

Gastrula

You can recognize the gastrula by the presence of a *crescentric slit*. This is the blastopore, where cells are invaginating. The blastopore later takes on a circular shape, but is *plugged* by yolk cells that do not invaginate. In the frog, invagination is accomplished primarily by yolkless cells from the animal pole. We also see the influence of a moderate amount of yolk in the formation of the mesoderm. This germ layer develops by invagination of cells at the dorsal lip of the blastopore. The inert yolk cells, which fill up half of the blastocoel, do not allow outpocketing to occur. Gastrulation is complete when there is a 3-layered embryo.

Neurula Stage

On the dorsal surface of the embryo, 2 folds of ectoderm grow upward as the neural folds; the flat layer of ectoderm between them is the *neural plate;* the tube resulting from closure of the folds is the nerve cord. An examination of the embryo in cross section shows that the nervous system develops directly above the *notochord,* a structure that arises from mesoderm in the middorsal region.

S 1. Sketch this stage in the space provided at the end of this section.
S 2. Write the name of the stage below the sketch.
S 3. Label the *neural plate* and *notochord*.

Later Stages

Later stages may be available.
Follow your instructor's directions, which will tell you what stages are available and which structures to look for and label.

Induction

The notochord is said to *induce* the formation of the nervous system. Experiments have shown that if contact with notochord mesoderm is prevented, no neural plate is formed. Even more dramatic are experiments in which presumptive notochord is transplanted so that it underlies an area of ectoderm not in the dorsal midline. This ectoderm, which normally does not do anything of the kind, is induced to differentiate into neural plate tissue.

Induction is believed to be one means by which development proceeds in an orderly way. The part of the embryo that induces the formation of an adjacent organ is said to be an organizer and is believed to carry out its function by the release of (a) chemical substance(s).

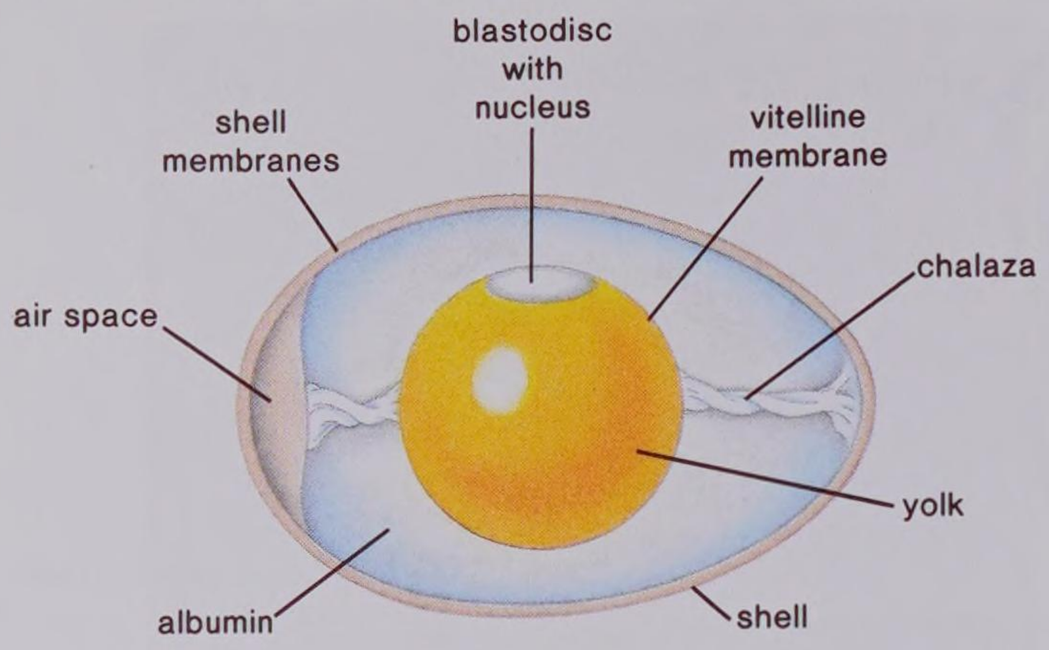
Germ Layers

Our study thus far has shown us that early development proceeds to the formation of 3 germ layers.
Ⓢ In table 30.1, indicate the *germ layers* and the *major organs* that develop from each, as discussed in the introduction.

Table 30.1 Germ Layer Organization

Germ Layers	Organs
1.	
2.	
3.	

Figure 30.3 Internal structure of chick egg.



Chick Development

Unfertilized Eggs

1. Obtain a 5-in finger bowl and carefully crack an unfertilized egg into the bowl.
2. Examine the contents of a raw egg (fig. 30.3). Note that some of the egg white (*albumin*) is denser and thicker than the remainder; these thicker masses are the *chalazae,* 2 spirally wound strands of albumin.
3. On the top of the yolk mass, note the *blastodisc,* a small white spot containing the clear cytoplasm of the egg cell and the egg nucleus. Only the yolk with its blastodisc is the ovum. The various layers of albumin, including the chalazae, the shell membranes, and the shell, are all secreted by the oviduct as the ovum travels down it.

Live Chick Embryos

When a fertile egg is freshly laid, the chick embryo has already begun the process of gastrulation. Because of the very large amount of yolk, the embryo lies open at first on the yolk. The cleavage of the blastodisc has produced a *blastoderm,* the equivalent of the blastula in the frog. Endodermal cells have begun to segregate as a lower layer of the blastoderm through the process of *delamination* (a separation of cell layers by splitting). Cells of the outer layer of the blastoderm begin to converge on the posterior midline of the embryo, invaginating through an anteroposterior groove called the *primitive streak* and spreading laterally between the inner and outer layers of cells of the blastoderm (fig. 30.4). Mesoderm cells invaginate through the anterior end of the primitive streak, forming the primitive pit (or Henson's node), and push anteriorly to form the notochord.

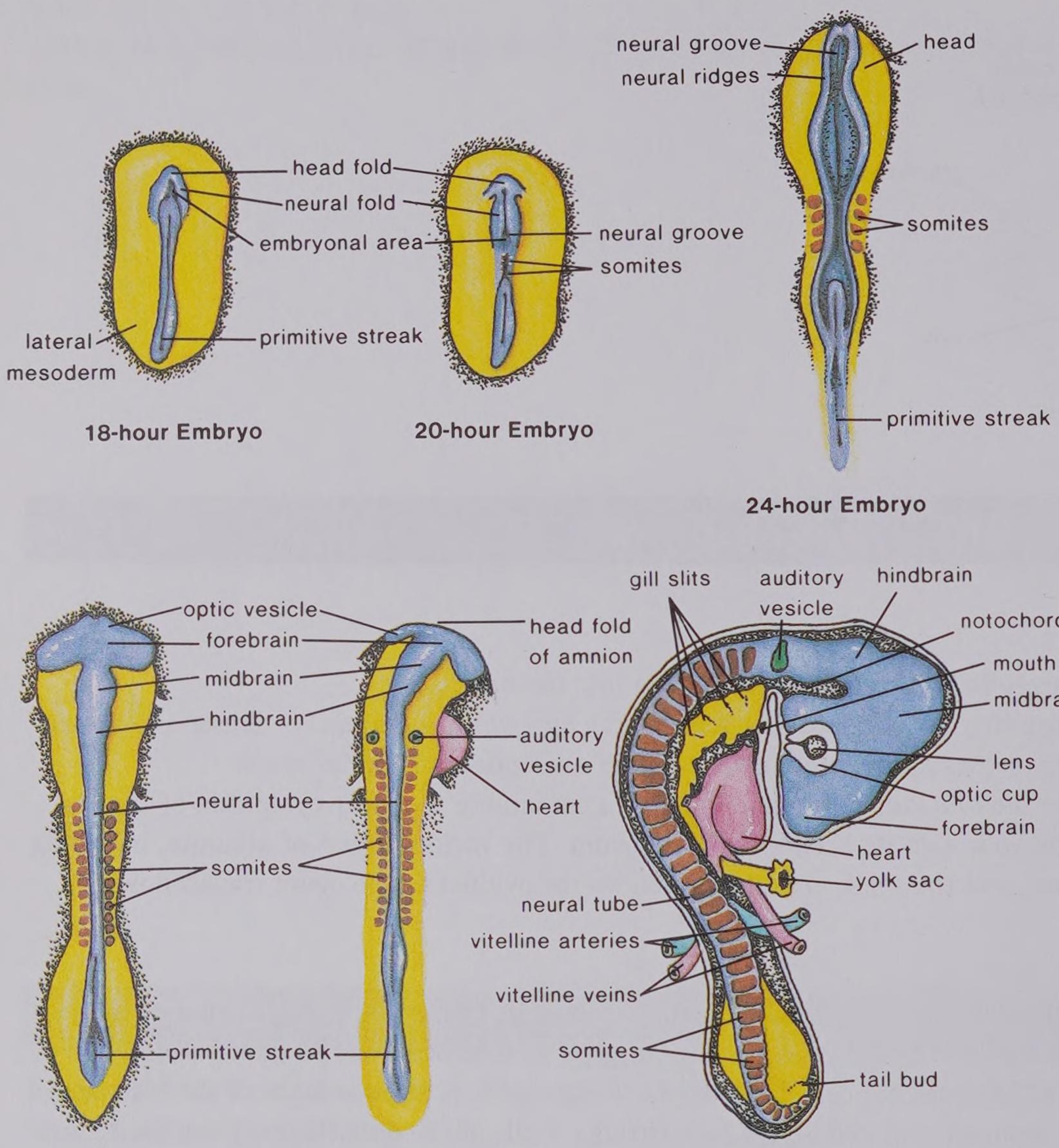
Once the 3-layered condition is established, the mesodermal layer splits to form the body cavity or *coelom,* as in the frog, and morphogenetic processes involved in organ formation become evident.

38-Hour Chick (fig. 30.4)

An embryo at this stage of development is already well beyond gastrulation. The blastoderm has become a thin disc about ¾ inch in diameter. Surrounding the embryo is a transparent region (the *area pellucida*). Outside of this is the *area opaca,* where the blood vessels are beginning to develop. One of the most striking features of this stage is the heart, which can be seen lying on the right side of the embryo. A pair of large *vitelline veins* enters the heart posteriorly and a single ventral aorta leaves it anteriorly. The vitelline veins carry food material from the yolk mass to the embryo.

Note that the head region of the embryo is divided into 3 segments: an anterior *forebrain,* a median enlargement (the *midbrain*), and finally the *hindbrain,* which continues as the neural tube for the length of the embryo. The outline of the *foregut* is clearly visible on either side of the midbrain area. The notochord can be seen as a wide band in the middle portion of the embryo and extending forward to the forebrain as a solid rod. Lying on either side of the neural tube is a series of 12–16 somites.

Figure 30.4 Stages in development of the chick.



72-Hour Embryo (figs. 30.4 and 30.5)

At this stage, the anterior part of the embryo has turned so as to lie upon its left side, while the posterior half remains dorsal side up. Note the 3 pairs of *gill slits* and the *aortic arches* that pass through the gill arches. The central nervous system and the sense organs have advanced considerably beyond their development in the 38-hour stage. The neural tube has developed into a 5-part brain anteriorly and the spinal cord posteriorly. The optic vesicles have greatly enlarged and by invaginating have given rise to the double-walled *optic cups*. Concurrently, the surface ectoderm of the head immediately over the optic cups invaginates, thickens, and detaches to form the *optic lens*.

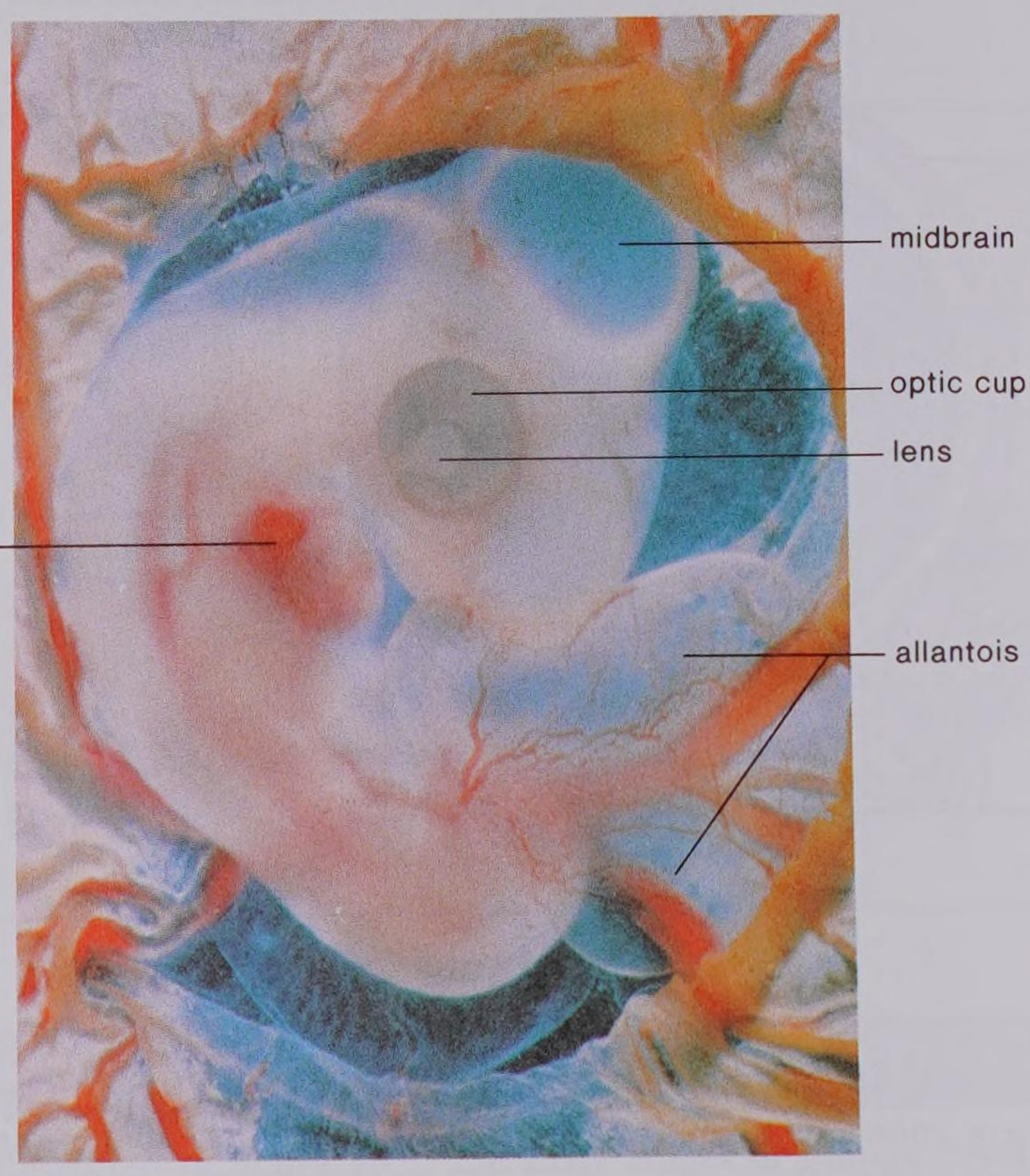
Dorsal to the gill slits on each side is an invaginated thickening of the ectoderm forming the *auditory vesicle*. The heart, which was tubular and single-chambered in the earlier stage, has become twisted and transformed into a 2-chambered structure in this stage. That part of the heart next to the body is the auricle, and the part that dips ventrally is the ventricle. Note the *truncus arteriosus;* it arises from the ventricle and branches out into the aortic arches, which unite to form the dorsal aortae. Note the *vitelline arteries,* a pair of large transverse blood vessels that leave the embryo about a third of the distance from the posterior end and branch out into the yolk sac. Note the vitelline veins that enter the auricle.

38-Hour Chick Observations

Ⓢ 1. Prepare a holder for an incubated egg as directed by your instructor.

Ⓢ 2. Obtain an egg that has been incubated for 38 hours, pick up the egg carefully, and place it in the holder. *Do not shake or rotate the egg.*

Figure 30.5 Chick embryo, 72 hours.



3. Poke a hole with the point of a forceps; do not penetrate the shell membrane.
4. Carefully remove the shell piece by piece from the top of the egg.
5. With the point of the scissors, puncture the shell membrane and carefully remove with forceps.
6. Examine the embryo, and with the aid of figure 30.4, identify as many of the structures as you can.
7. Write the names of the visible structures in the space provided for the 38-hour embryo.

_____

_____

72–96-Hour Chick Observations

1. Obtain an egg that has been incubated for 72–96 hours.
2. Open the shell as directed previously.
3. Examine the embryo, and with the aid of figures 30.4 and 30.5, identify as many of the structures as you can.
4. Write the names of the visible structures in the space provided for the 72–96-hour embryo.

_____

_____

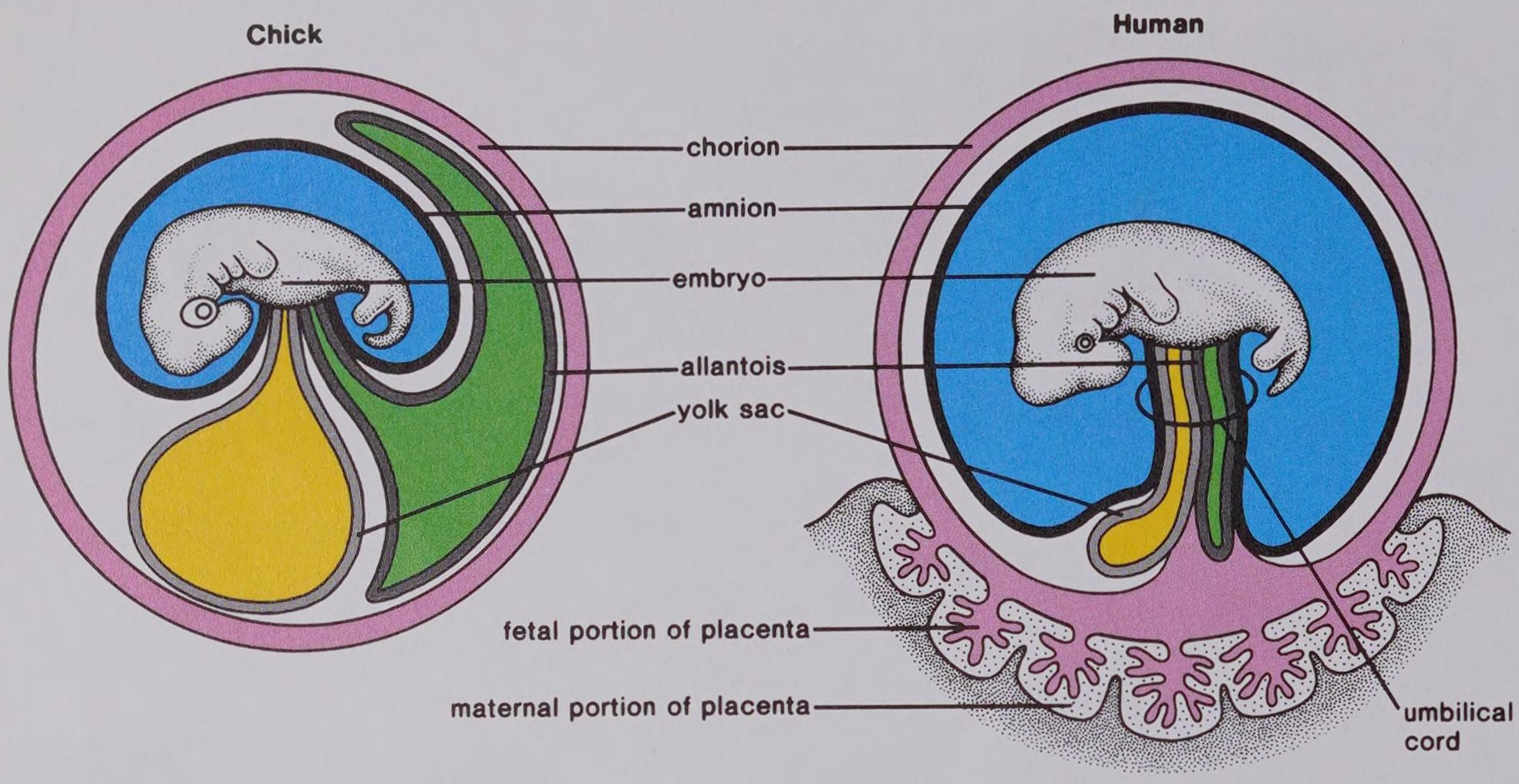
Eggs from 5–10 days may be observed by breaking away pieces of the shell without damaging the fragile, highly vascular underlying membranes. Or you may cut a window in the shell with a fine-toothed hacksaw blade. Open this egg carefully to observe the movements of a living embryo. If you watch closely, you should see body movements, head motion, and limb bud activity.

Eggs from 11–21 days may be examined by simply breaking the shell, putting the embryo in a petri dish with Ringer's, and removing all extraembryonic membranes (see next section).

5. List the age of any available older eggs and any structures not previously seen. _____

_____

Figure 30.6 Comparison of the extraembryonic membranes in the chick (***a***) and mammal (***b***).



Extraembryonic Membranes

The embryos of land vertebrates (reptiles, birds, and mammals) are surrounded and covered by membranes that do not become part of the animal. Therefore, these membranes are known as *extraembryonic membranes* (fig. 30.6). The extraembryonic membranes are the *amnion, chorion, allantois,* and *yolk sac*. The *chorion* is the outermost membrane. In chicks, it lies just below the porous shell, where it functions in gas exchange. In mammals, such as humans, it forms the fetal portion of the placenta through which nutrients, gases, and wastes are exchanged with the blood of the mother.

The *amnion* is a delicate membrane that contains the amniotic fluid that bathes the embryo. The embryo, thus suspended in a fluid medium, is protected from mechanical shocks. The *yolk sac* surrounds the yolk in bird and reptile eggs but is largely nonfunctional in mammals. The *allantois* serves as a storage area for metabolic waste products in reptiles and birds. In mammals, the allantoic blood vessels are part of the placenta.

Study the models of human development, and distinguish as many extraembryonic membranes as possible.

S 1. List the observed membranes in the following space.

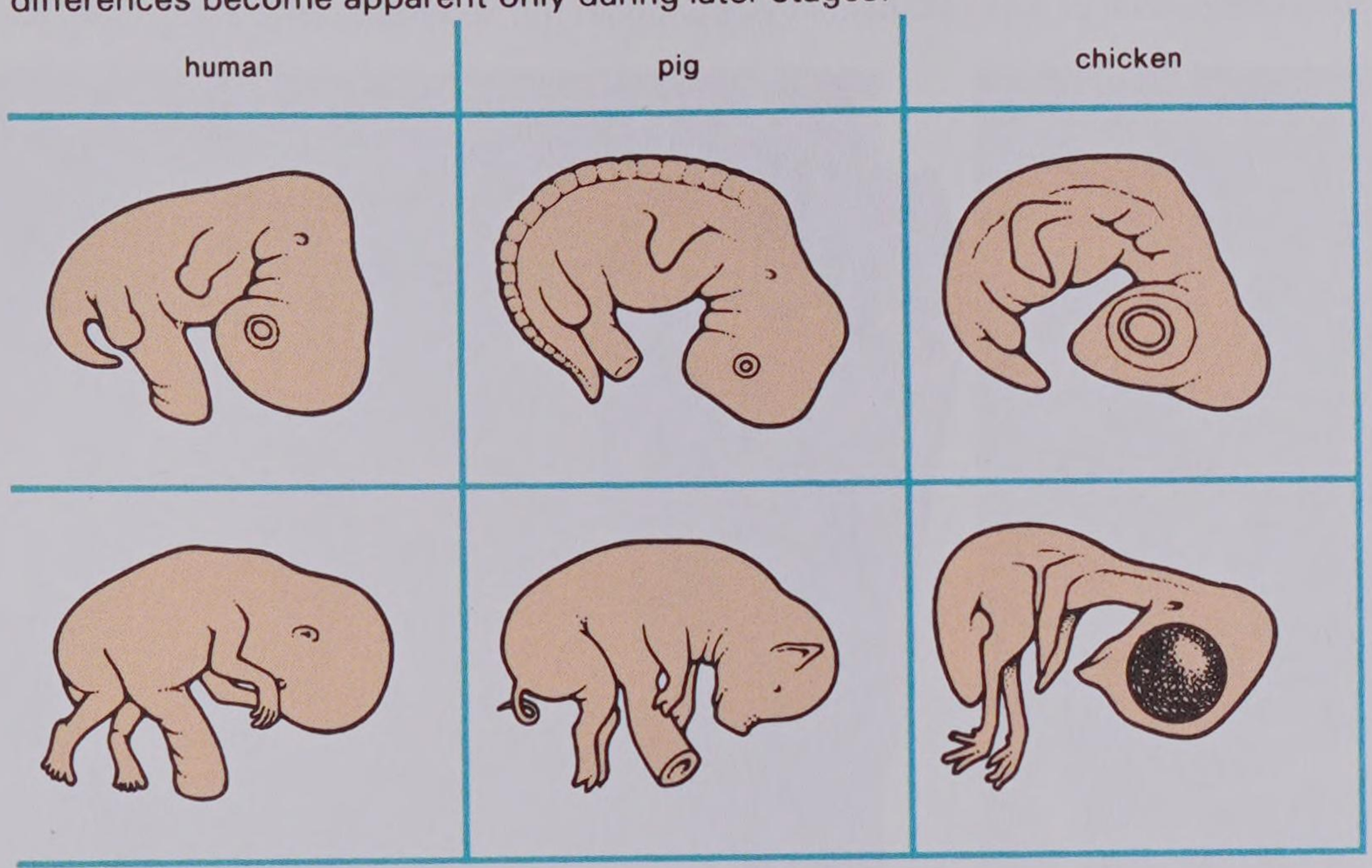
_____

S 2. Using the preceding description, identify the differences between the various extraembryonic membranes in bird and mammals and fill in table 30.2.

Table 30.2 Extraembryonic Membranes—Differences and Similarities

Membrane	Mammal	Bird/Reptile
Amnion		
Chorion		
Allantois		
Yolk sac		

Figure 30.7 Successive stages in the embryonic development of chick, pig, and human. Early stages are similar; differences become apparent only during later stages.



Human Development

As illustrated in figure 30.7, the early stages of human development are quite similar to those of the chick and pig. Differences become marked only as development proceeds.

Stages

Human development is divided into 2 portions:

Embryonic development (first 2 months)

First the germ layers and extraembryonic membranes develop; then the internal organs form. At the end of this period, the embryo takes on a human appearance.

Fetal development (last 7 months)

The skeleton becomes ossified (bony), reproductive organs form, arms and legs fully develop, and the fetus enlarges in size and puts on weight.

S 1. Compare the illustrations (figs. 30.8, 30.9, 30.10, and 30.11) to your models and try to determine the age of the models.

S 2. List the ages of your models. _____

Figure 30.8 Human development, early embryonic. ***a.*** Sixteen-day human embryo. ***b.*** Twenty-nine-day human embryo.

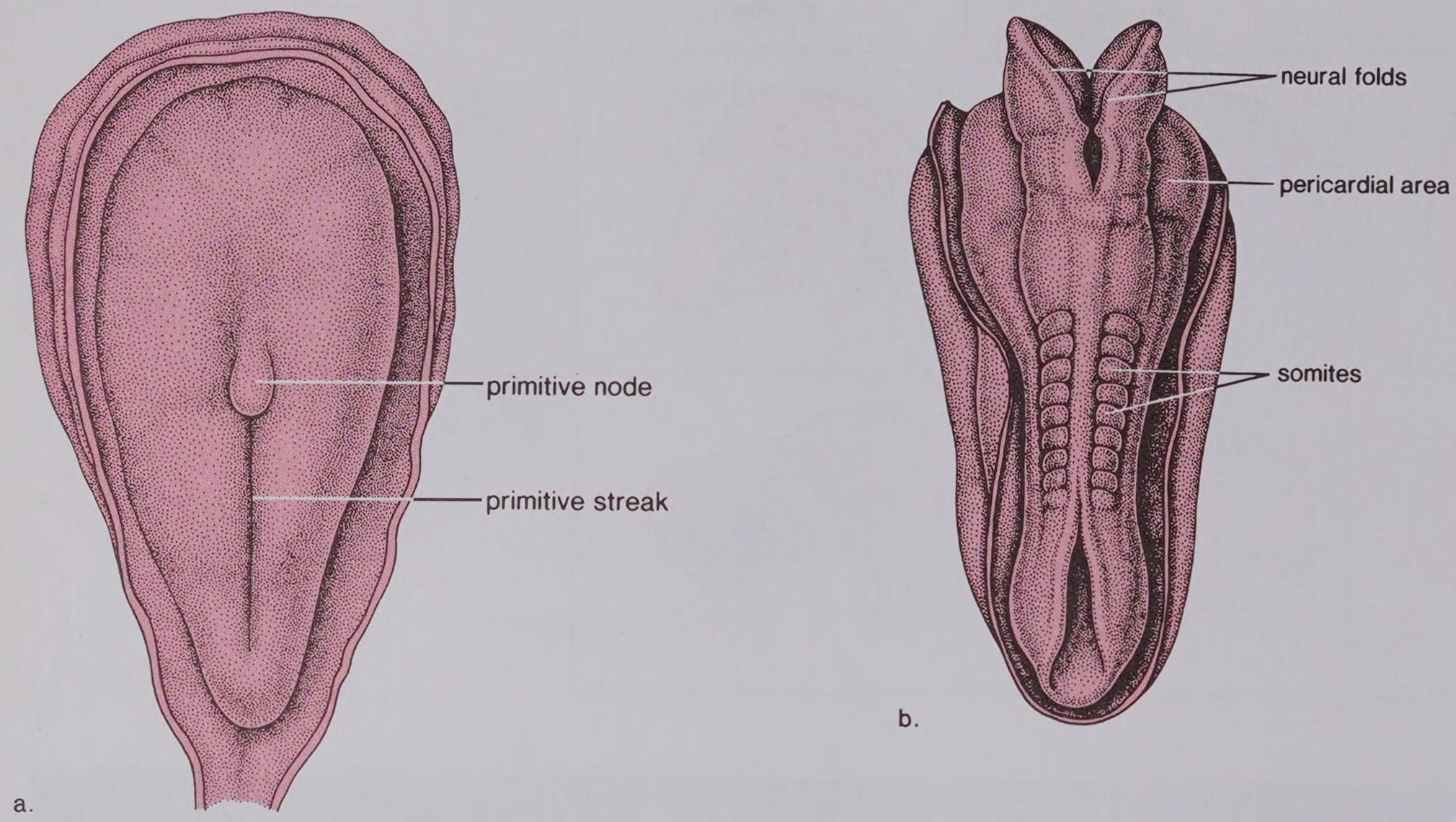
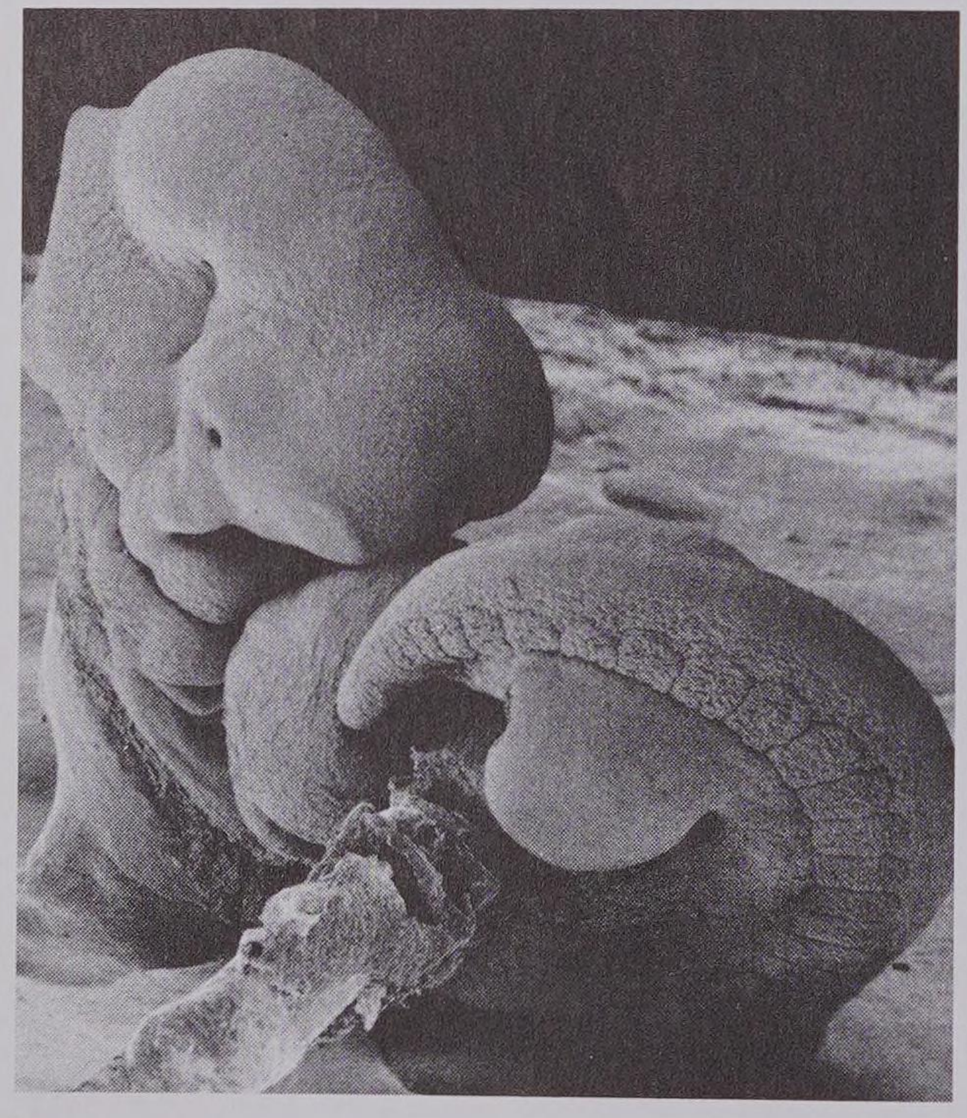


Figure 30.9 Human embryo at beginning of fifth week. ***a.*** Scanning electron micrograph. ***b.*** Drawing. The embryo is curled so that the head touches the heart, 2 organs whose development is further along than the rest of the body. The organs of the gastrointestinal tract are forming. The presence of the tail is an evolutionary remnant; it will later regress. The arms and legs will develop from the bulges that are called limb buds.



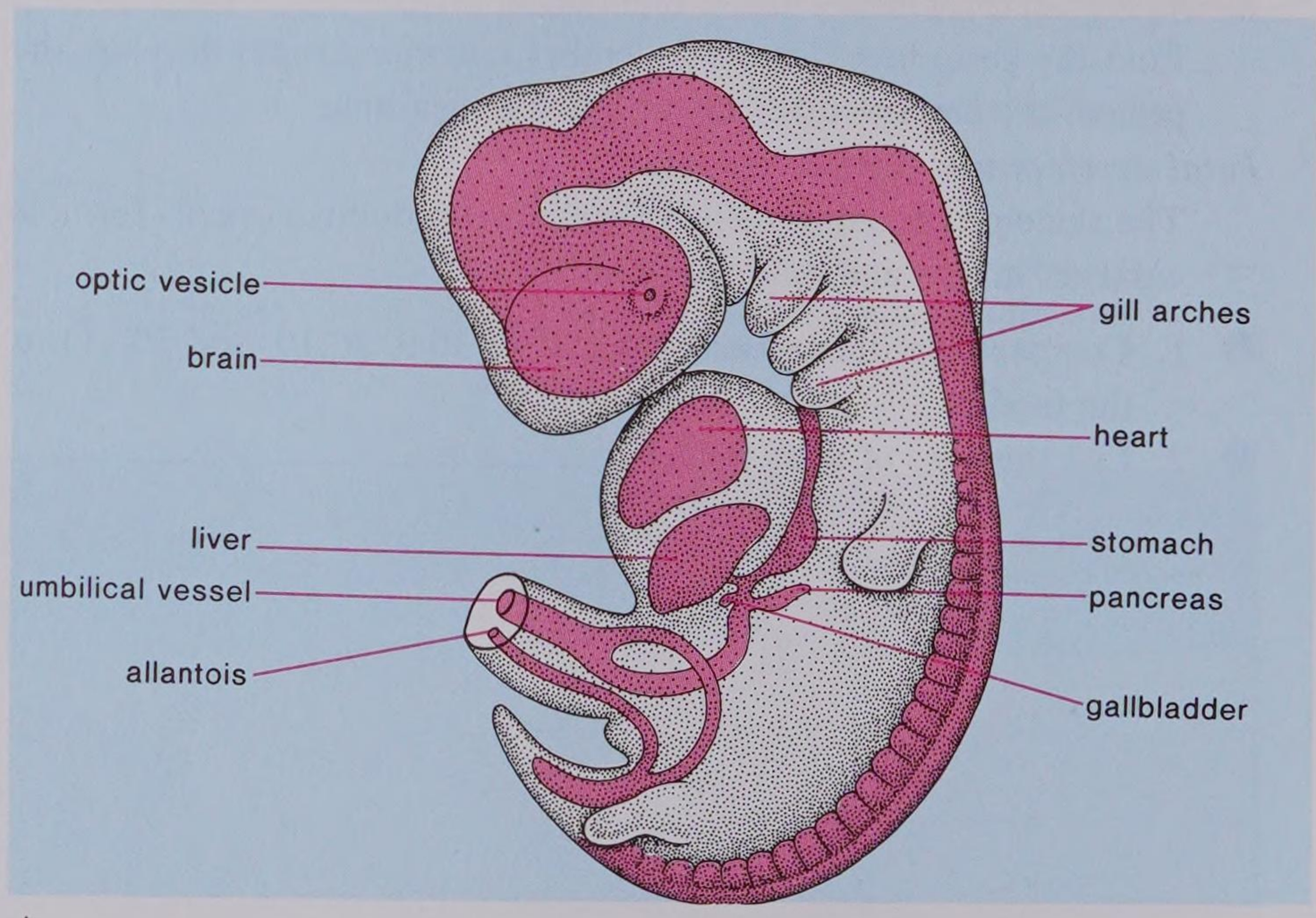
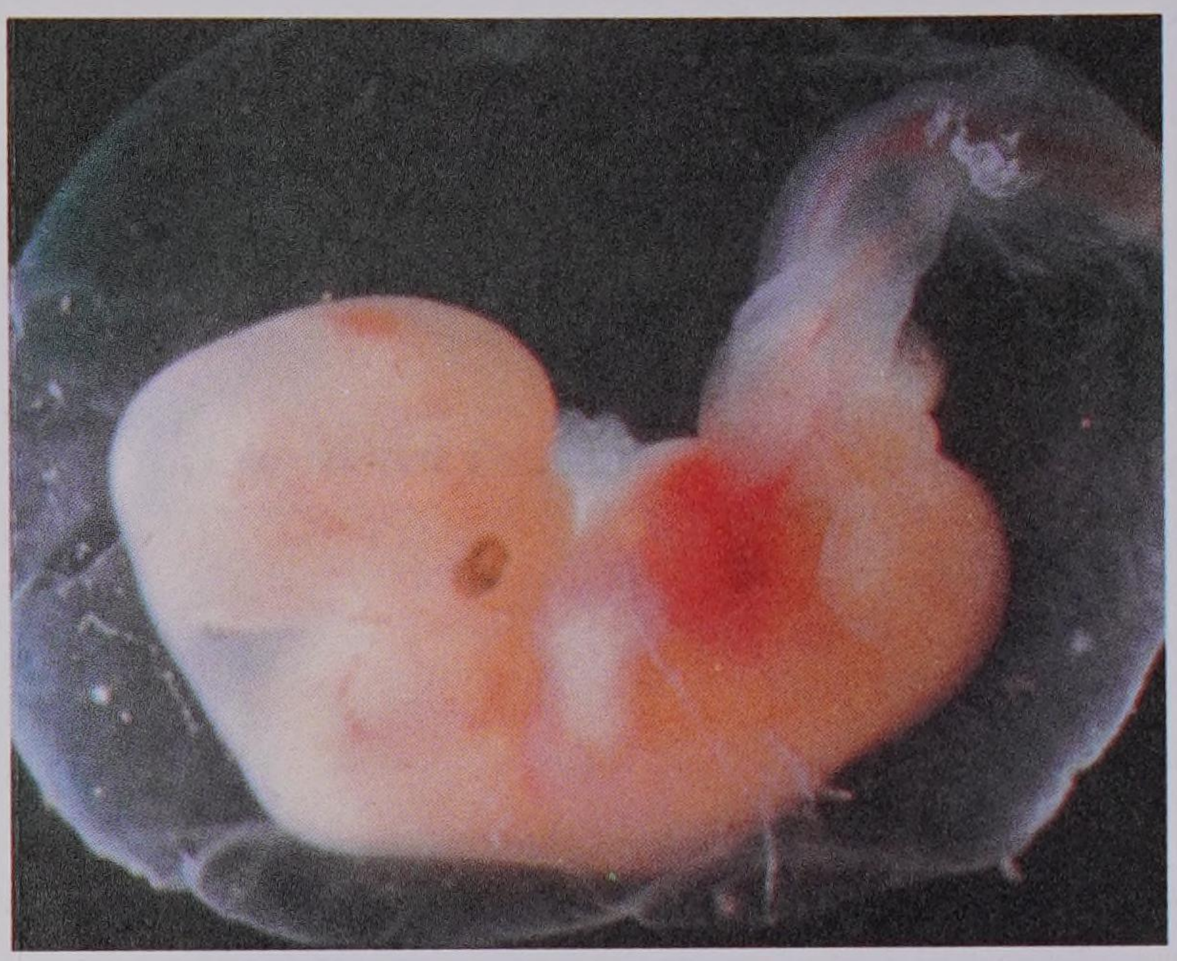


Figure 30.10 Human development is divided into the embryonic period (first 2 months) and fetal development (third to ninth month). ***a.*** Embryo is not recognizably human. ***b.*** Fetus is recognizably human.



a.

b.

Figure 30.11 Early fetal development: 3–4 months old.

Laboratory Review 30

_____ 1. Name the stage of development when the embryo is a solid ball of cells.

_____ 2. From the formation of the zygote to the formation of the gastrula, there is little increase in the size of the embryo. What happens to the number and the size of the cells during this time?

_____ 3. Name the stage of development when the germ layers are forming.

_____ 4. What term refers to the last 7 months of human development?

_____ 5. What is the specific function of the amnion?

6. What are the 3 primary germ layers found in developing embryos?

_____

_____

_____

7. Name several structures that each primary germ layer gives rise to in the adult organism.

8. Would you be more likely to find extraembryonic membranes in an aquatic animal or a land animal? Why?

Reference

Paul, C. 1972. *Principles of Biological Science.* Dubuque, IA: Kendall/Hunt Publishing Company.

Laboratory

31

Animal Behavior

Learning Objectives

Students should be able to

1. describe the experiments in this laboratory exercise and state their purpose;
2. contrast the behavior of a planarian with a fruit fly to the degree studied.

Introduction

Behavior is exemplified by an organism's routine activities. Figure 31.1 suggests that behavior occurs as a response to a stimulus. The specific activity, as the animal responds to the stimulus, is dependent on the anatomy and physiology of the organism. In other words, the *limits of the organism* determine the manner in which the organism responds. The diagram also suggests that the internal readiness of the organism influences whether the animal responds to the stimulus.

Behavior can be *innate* or *learned*. The proportion of innate and learned behavior common to an animal is dependent on the complexity of the nervous system. Figure 31.2 shows that innate behavior is common to *lower* organisms, while learned behavior is common to *higher* organisms. Today we are going to study taxes and the feeding reflex in 2 invertebrates, the fruit fly and the planarian. A *taxis* is an orientation of the body toward or away from a stimulus. A *reflex* action is an automatic rapid response to a stimulus. We are also going to study an instinctive behavior in fish, which are vertebrates. *Instinctive behavior* also occurs automatically and need not be learned. The behavior often requires a series of reflex actions.

Figure 31.1 Diagram to show that behavior is a response to a stimulus. The central nervous system CNS acts as the receiver and integrator of both external and internal stimuli.

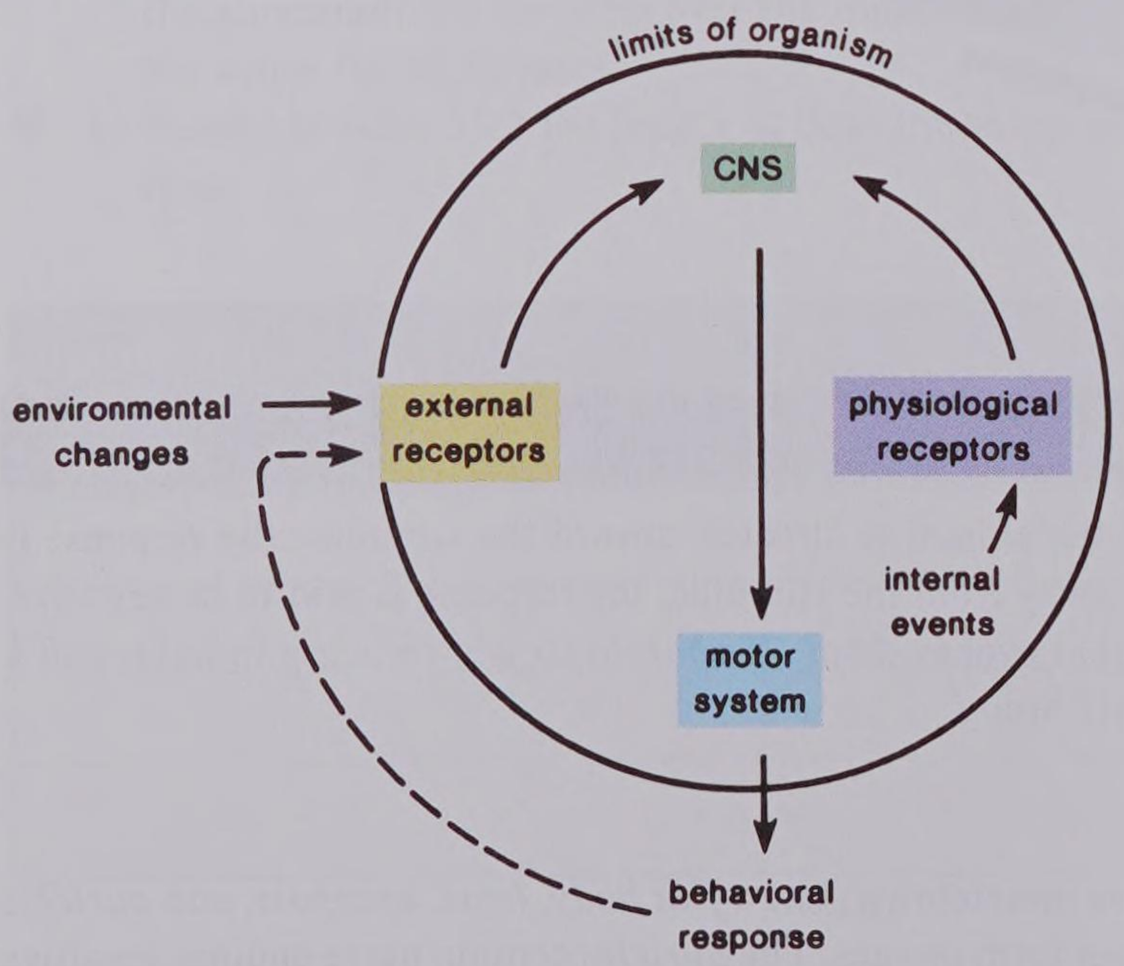
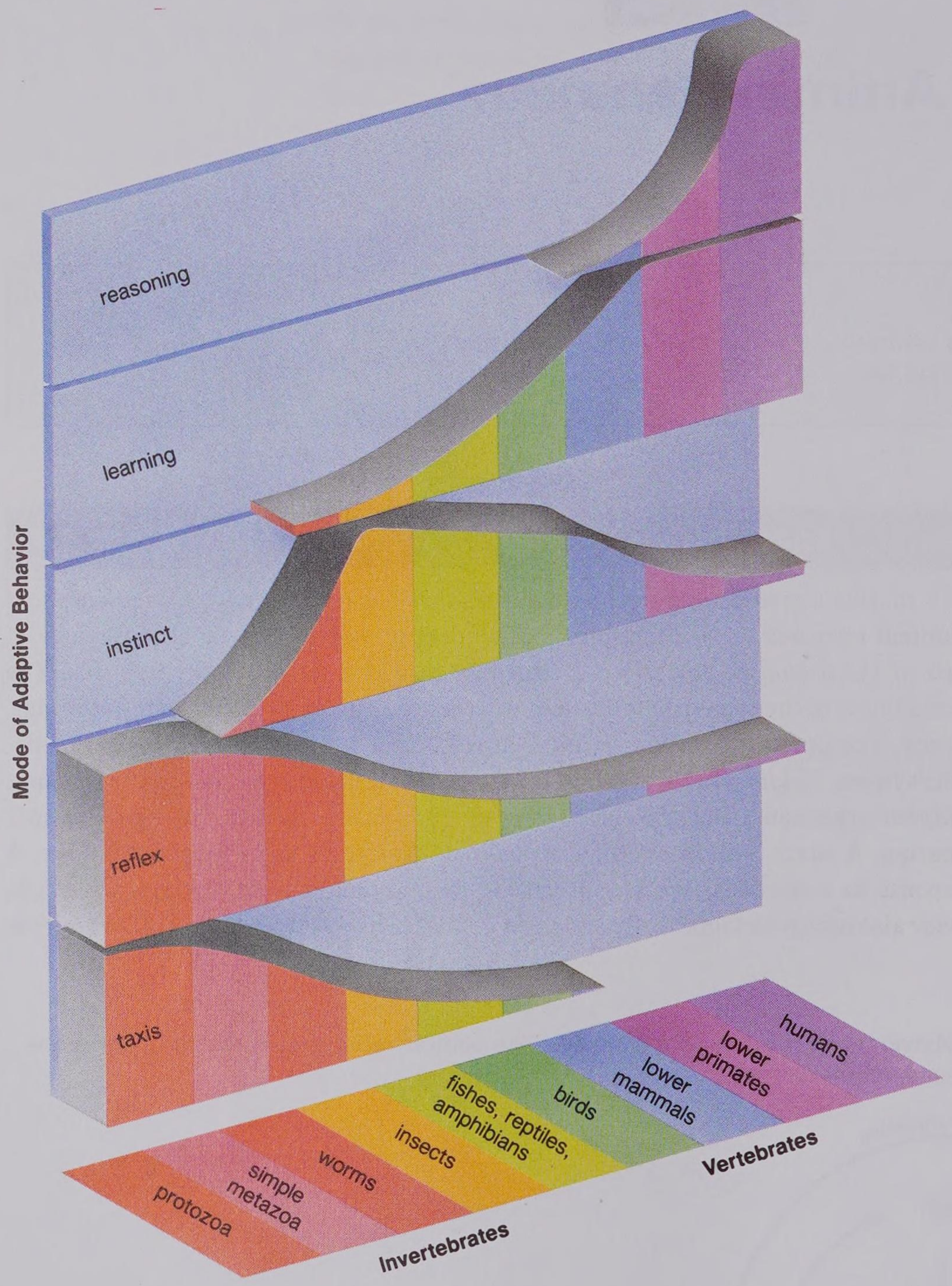


Figure 31.2 Comparative frequency of modes of adaptive behavior among invertebrates and vertebrates. *Innate* types of behavior (taxes and reflexes) are more frequently found among invertebrates, and *learned* types are more frequently found among vertebrates. Source: Data from V. G. Dethier and Eliot Stellar, *Animal Behavior,* Third Edition, © 1970, p. 91.



Taxes

Taxes are categorized in at least 2 ways. When the body of the animal is directed toward the stimulus, the response is said to be *positive.* When the body of the animal is directed away from the stimulus, the response is said to be *negative.* Furthermore, responses are named for the type of stimulus that evokes them. A *phototaxis* is a response to light, and a *geotaxis* is a response to gravity. These are the 2 taxes we will study.

Planarians

The flatworm *Planaria* (phylum Platyhelminthes), a primitive invertebrate, has a *flat body, head, eyespots,* and *auricles* at the sides of the head. The *eyes* are sensitive to light but do not form images. The *auricles* contain nerve endings sensitive to touch and chemicals. The *mouth* is located at the end of the *pharynx,* which can be extended from the midventral surface of the body when a planarian is feeding.

Geotaxis

1. Obtain a small test tube and cork stopper.
2. Draw a line around the test tube at the midpoint.
3. Transfer a flatworm from the culture bottle to your tube by means of a medicine dropper, as quickly as possible. If the worm gets stuck in the dropper, insert a dissecting needle into the open end of the dropper.
4. Dislodge the worm as you squirt water into the dropper. As soon as you have the worm in your tube, fill it with culture water (or distilled water) and then stopper it.
5. Hold the test tube horizontally until the flatworm has moved to the middle of the tube. Lighting should be subdued and even from all angles.
6. Place the tube in a test tube rack or a beaker (stopper up) and note the time.
7. Watch the worm for 10 minutes and record in table 31.1 the length of time the worm spends in the top half (above the marker) and the bottom half of the tube.

Table 31.1 Geotaxis in Planarians

Actual Time	Top Half (length of time)	Bottom Half (length of time)
	Total:	Total:

Does the planarian demonstrate geotaxis? _____ Negative or positive? _____

8. Check your results with those of others. _____

Phototaxis

1. Obtain a piece of aluminum foil and mold a covering for the rounded half of the tube.
2. Take the covering off the tube and place the tube on a piece of white paper beneath a lamp. If it is not level, adjust the height of one end by using aluminum foil, glass slides, and so forth, until it is even.
3. After a few minutes, when the planarian is in the center of the tube and headed toward the stoppered end, slip the aluminum foil covering over the rounded end of the test tube. Turn on the lamp, note the time, and watch the worm for 10 minutes.
4. Record in table 31.2 the length of time the worm spends in the lighted half and in the darkened half of the tube.

Table 31.2 Phototaxis in Planarians

Actual Time	Lighted Half (length of time)	Darkened Half (length of time)
	Total:	Total:

Does the planarian demonstrate phototaxis? _____ Negative or positive? _____

5. Check your results with those of others. _____

_____

By what anatomical means does the planarian detect light? _____

Fruit Flies

Geotaxis

1. Obtain a tube, plugged at both ends, that contains a number of adult *Drosophila* fruit flies.
2. Draw a circle around the tube at the midpoint.
3. Count the number of flies in the tube.
4. Tap the tube lightly to bring the flies to one end. Lighting should be subdued and even from all angles.
5. Hold the tube upright and observe the reaction of the flies.
6. Count the number of flies above and below the drawn circle. Calculate the proportion of flies at each end and record your data in table 31.3.
7. Invert the tube and repeat your observations.

Table 31.3 Geotaxis in Fruit Flies

	Proportion Showing Positive Geotaxis	Proportion Showing Negative Geotaxis
Trial 1		
Trial 2		

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Does *Drosophila* demonstrate geotaxis? _____ Negative or positive? _____

8. Check your results with those of others. _____

_____

Phototaxis

1. Obtain a Y-shaped tube containing a number of adult *Drosophila* fruit flies and a piece of aluminum foil.
2. Count the number of flies in the tube.
3. Shape the aluminum foil so that it shades one arm of the tube but you can still see the flies. Be sure the boundary between the light and dark portions of the tube is exactly in the middle of the fork.
4. Tap the tube gently to bring the flies into the stem of the tube.
5. Keep your left hand closed over the straight arm of the Y-shaped tube, in which the animals begin their movements, so that it will be dark.
6. Turn on the lamp and bring the tube near the bulb so that one arm is shaded by the aluminum foil.
7. Count the number of flies that go into the darkened tube and the number that go into the lighted tube.
8. Calculate the proportion of flies in each arm and record your data in table 31.4. (Disregard any flies that do not go into an arm.) Repeat the test.

Table 31.4 Phototaxis in Fruit Flies

	Proportion Showing Positive Phototaxis	Proportion Showing Negative Phototaxis
Trial 1		
Trial 2		

Does *Drosophila* demonstrate phototaxis? _____ Negative or positive? _____

9. Check your results with those of others. _____

_____

Comparison

Compare planarians with fruit flies in table 31.5.

Table 31.5 Comparison

	Geotaxis	Phototaxis
Planarian		
Fruit flies		

1. Look again at figure 31.1 and notice that the *limits of the organism* affect behavior. For example, the anatomy of an organism determines the manner in which behavior takes place.
2. Compare the manner in which a planarian responds to a stimulus, such as gravity and light, to the manner in which a fruit fly responds to these stimuli. _____

_____

_____

Reflexes

The sucking reflex in a newborn infant is automatic; when a nipple is placed in the mouth, the newborn immediately begins to suck. Feeding behavior in planarians and fruit flies also appears to be automatic. What stimulus triggers the reflex?

_____

Planarians

1. Obtain a watch glass or petri dish.
2. Fill it half full with *culture water*. Transfer a planarian from the culture bottle to the container. The container should be on a piece of white paper, and the lighting should be diffuse.
3. Watch the worm for about 5 minutes, using a binocular dissecting microscope or a hand magnifying lens.
4. Put 2 or 3 very small pieces of raw beef or, preferably, raw liver as close to the center of the container as possible.
5. Watch the behavior of the worm (fig. 31.3).

   What part of the body seems to assist the worm in locating food? _____

   _____
6. Describe the behavior of the worm after it has located the food.

   _____

   _____

Figure 31.3 Feeding behavior of planarians, external view. ***a.*** When the worm is not feeding, the pharynx resides inside the pharyngeal chamber. ***b.*** When the worm is feeding, the pharynx is extended beneath the worm. (Copyright 1975 by Kendall/Hunt Publishing Company. Reprinted with permission.)

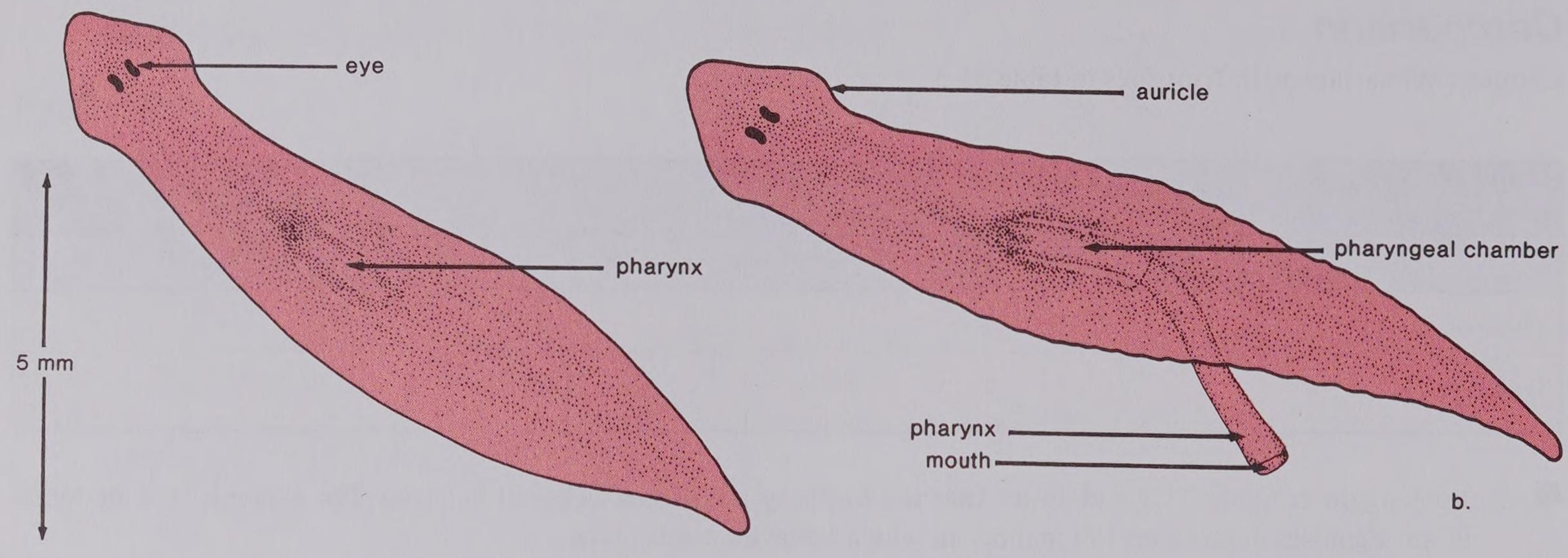
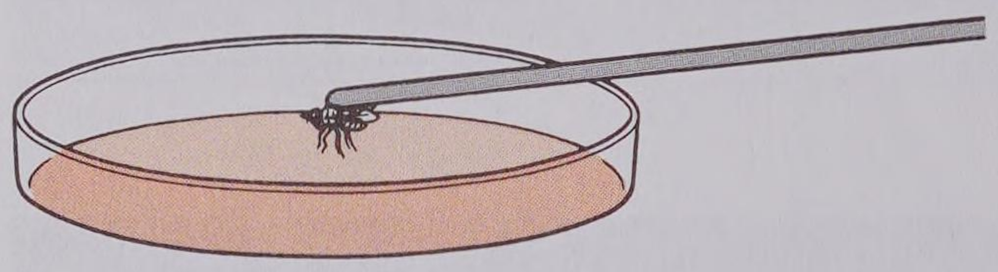


Figure 31.4 Fly attached to rod is lowered toward food until feet touch the surface.



Fruit Flies

1. Obtain a fly attached to a slender rod.
2. Gently lower a fly onto a source of food (crushed ripe banana, sugar water, prepared instant medium, etc.) in a petri dish until its feet touch the surface (fig. 31.4).
3. Observe the fly with a binocular dissecting microscope or a hand lens. In a fraction of a second, the *proboscis* will be extended (fig. 31.5).
4. Repeat the test several times until you are satisfied that the response is automatic.
5. Knowing that chemoreceptors are present on antennae, mouthparts, and legs of flies, explain how the fly might sense that food is present, causing the proboscis to be lowered.

_____

_____

Comparison

Compare the feeding behavior of a planarian with that of a fruit fly. _____

_____

_____

Does this comparison show that the *limits of the organism* affect behavior? _____

Study the reflex arc shown in figure 31.6, and then explain how feeding behavior could be a reflex action. _____

_____

_____

Figure 31.5 Feeding behavior of a fly. ***a.*** Proboscis retracted. ***b.*** Proboscis fully extended.

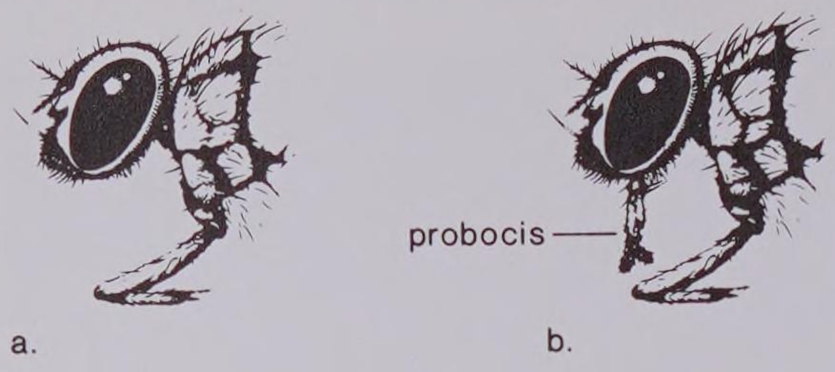
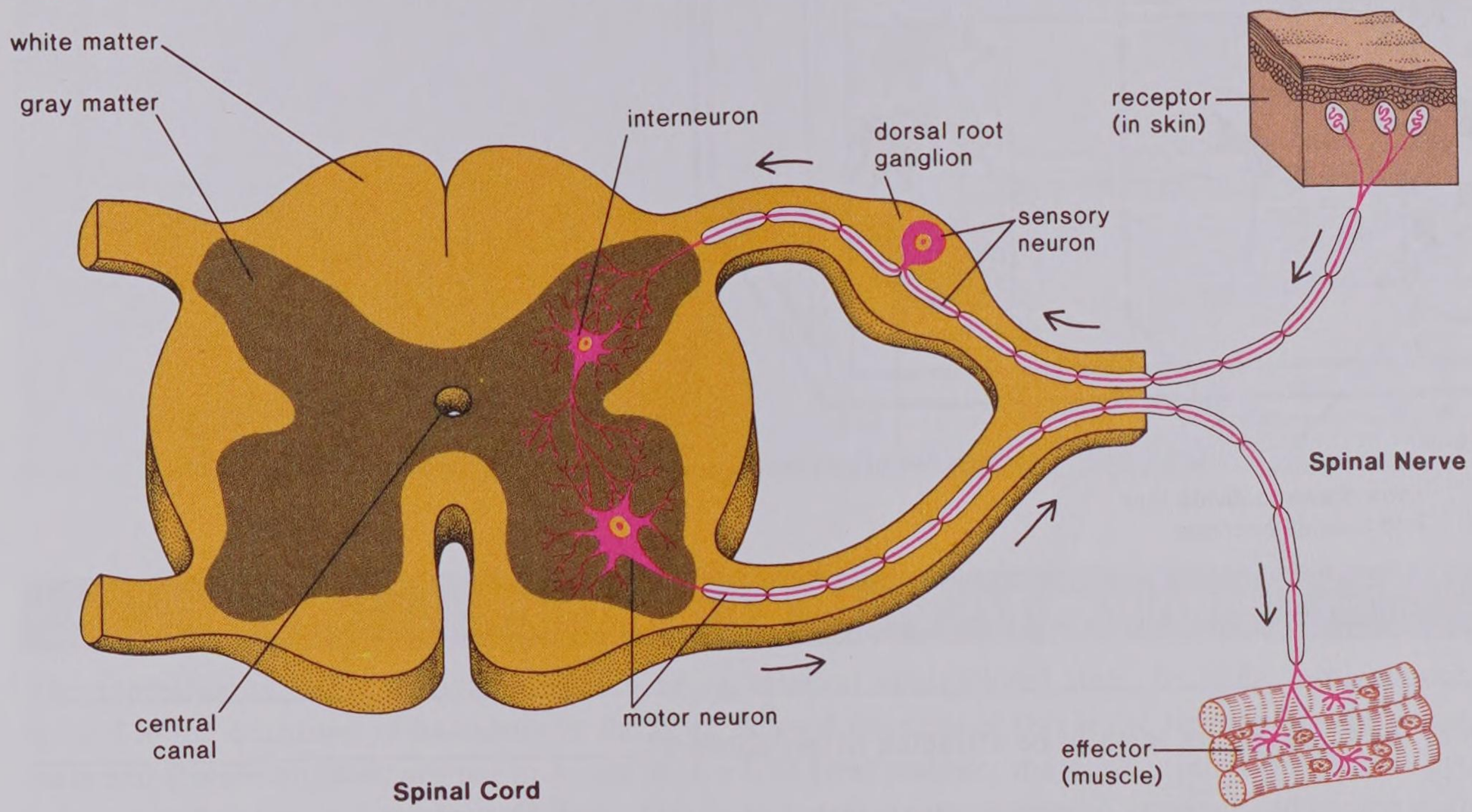


Figure 31.6 Spinal cord and spinal nerve. The arrows mark the path of a reflex arc.



Instinctive Behavior in Fish (Optional)

Fish swimming together in a school exhibit a type of behavior that borders on social behavior, especially since they are believed to be responding to each other rather than to some external stimulus. If this is the case, we would expect a fish to be attracted to members of its own species, a hypothesis we will test.

Trial 1

1. Draw 2 vertical lines on the front wall of a 15-gallon aquarium, as shown in figure 31.7.
2. Place equal numbers of 2 species of fish in separate 4-liter jars.
3. Place the jars at opposite ends of the aquarium. Three students, each with a stopwatch, will be assigned to watch a particular section of the aquarium for 10–15 minutes and keep track of the amount of time a test fish is in its third of the tank. These students are also responsible for watching the behavior of the fish carefully.
4. Gently place a single fish of the species to the right in the center of the aquarium and begin the timing.
5. Record the results in the space provided. Answer these questions.

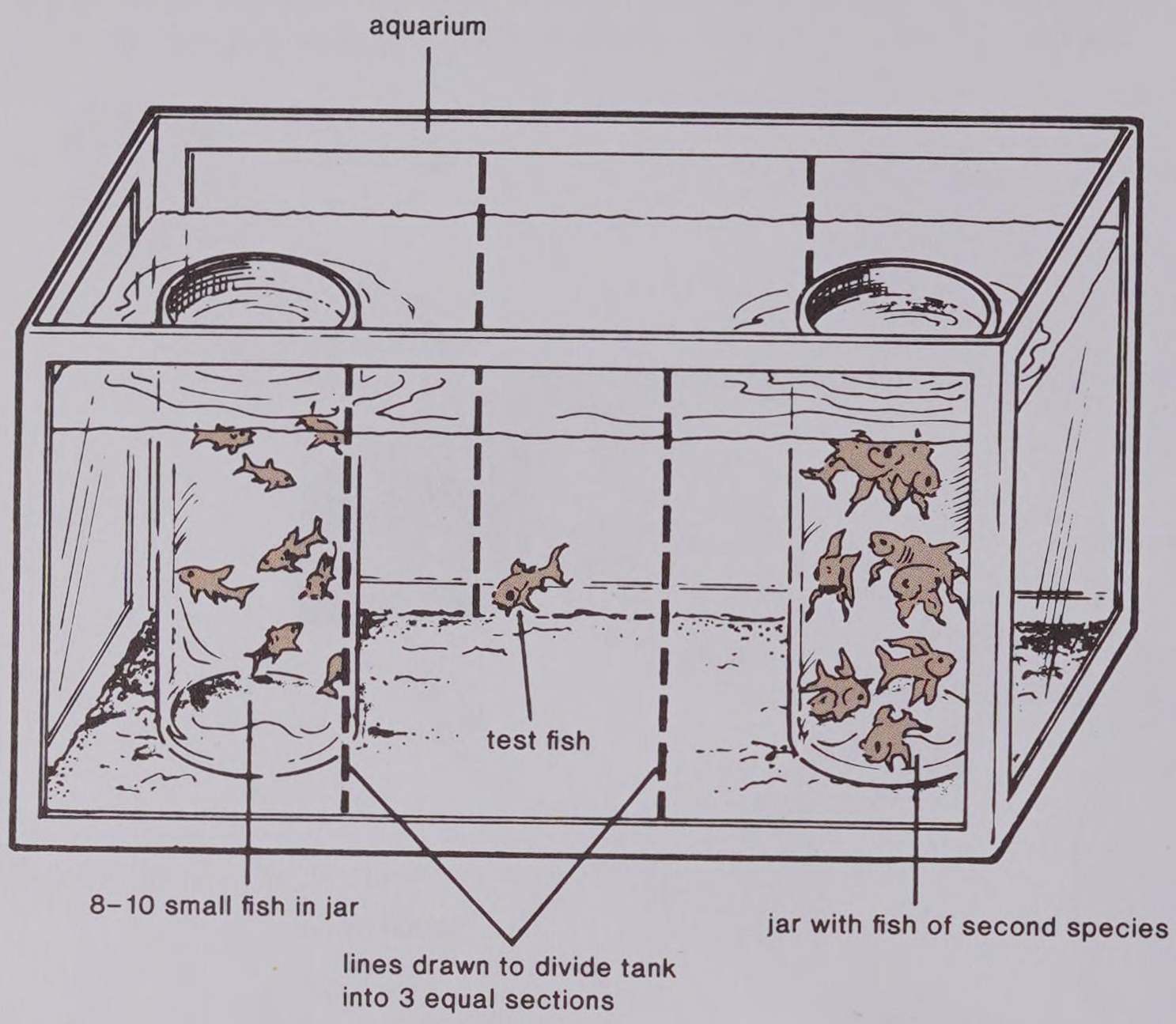
Name the species that was placed on the right? _____

Name the species that was placed on the left? _____

How did the enclosed fish react to the test fish? _____

_____

Figure 31.7 Procedure for testing schooling behavior in fish.



How did the test fish react to the enclosed fish? _____

_____

Did the fish of the same species seem to be attracted to one another? _____

Trial 2

1. Remove the first test fish, gently place a single fish of the other species in the center of the aquarium, and begin timing.
2. Record the results in table 31.6.
3. Answer these questions.

   How did the enclosed fish react to the test fish? _____

   _____

   How did the test fish react to the enclosed fish? _____

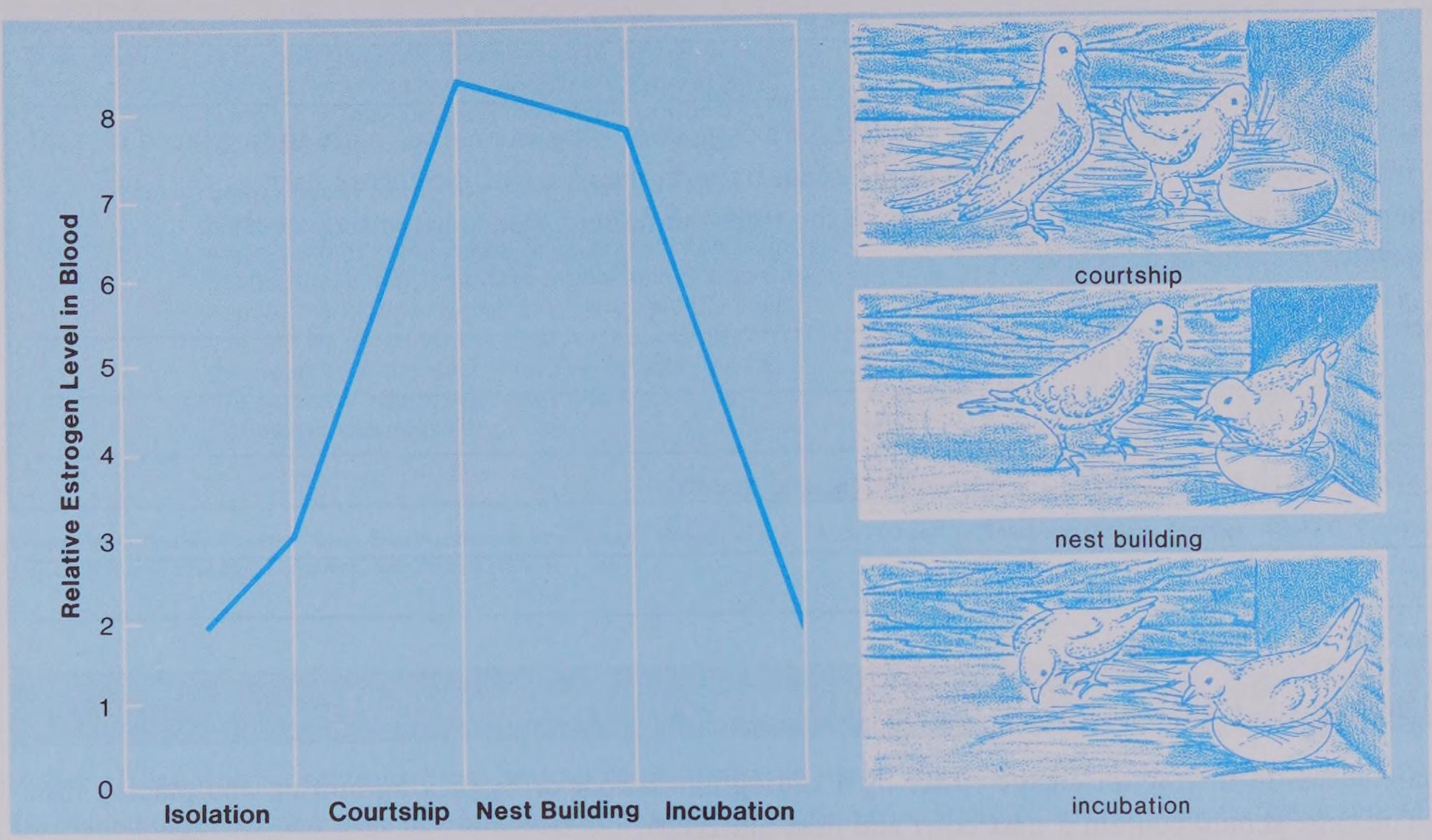
   _____

   Did fish of the same species seem to be attracted to one another? _____

Table 31.6 Schooling Behavior of Fish

	Time Right Third	Time Mid-third	Time Left Third
Trial 1 (same species to right)			
Trial 2 (same species to left)			

Figure 31.8 Changes in estrogen levels in female during different stages in the reproductive cycle of the ringdove.



Motivational State in Ringdoves

The expression of many behaviors depends on an internal motivational state. In birds, motivational states are closely related to the quantities of hormones in the body. A good example of this is the reproductive behavior of ringdoves. If a male and female ringdove are put in a cage with a nest bowl and nest material, a fairly predictable cycle of reproductive behavior will occur, lasting about 45 days. The male begins strutting around, bowing, and cooing in front of the female; then the pair begins to build a nest. During the course of nest building they copulate, and 7–11 days after the beginning of courtship, the female lays 2 eggs. When the chicks hatch, both parents feed them with crop milk that is produced by the lining of the crop (a sac in the first part of the bird's digestive tract). After 10 days or so, the chicks begin to feed on their own. When the young are between 2 and 3 weeks old, the adults begin a new cycle of reproduction.

It has been shown that the behavior of the female is related to the level of estrogen in the blood (fig. 31.8). It appears that the male's courtship display stimulates the female's ovaries to produce more estrogen. As estrogen production increases, she spends increasing amounts of time around the nest until, at peak estrogen level, she ovulates and lays eggs. When no nesting material is present, estrogen levels remain high, but the female will not ovulate. This shows that external stimuli as well as the internal physiological state are important to behavior.

Before observing a reproductive pair, we will make some preliminary observations. The laboratory members should be quiet during the observations and should sit or stand at least 2 feet away from the cages.

Male/Female Contact

Ⓢ 1. Place one female and one male in cages next to each other, with a visual partition between the cages. A large piece of oak tag serves as a good partition. Now they can react to each other only by sound.

Ⓢ 2. Observe for several minutes.

Ⓢ 3. Record your observations.

_____

_____

_____

4. Remove the partition, observe for several minutes, and record.

5. Place a visual partition on the opposite side of the female's cage and bring in another male to be placed on that side, so that his presence will only be sensed, but not seen, by both members of the future pair.
6. Watch for changes in the first male's behavior and in the time the female spends attending to each.
7. Observe for several minutes and record.

8. Remove the visual barrier, observe for several minutes, and record.

9. Return the second male to isolation.

Reproductive Behavior

1. Allow the male access to the female's cage either by a sliding hatch door or by picking him up and placing him in her cage. The cage should contain a nest bowl and nest materials.
2. Begin your observation as soon as you release him into her cage and record.

3. Return the male and female to their original cages and the cages to isolation.

Summary

Sum up the various behaviors you have seen by speculating on the functions of these behaviors (see tables 31.7 and 31.8).

Table 31.7 Qualitative Grouping of Behaviors (sample only)

Self-directed	Agonistic	Courtship	Parental
Drinking	Quick coos	Bow-coos	Incubating
Feeding/pecking	Bows	Ka-calls	Brooding
Walking	Pacing	Ring pecking	Feeding young
Preening	Semi-orientation	Mock-feeding	Preening young
Sleeping	Displacement activity	Chase	Maintaining nest
Perching	Fencing	Showing nest	
Flight	Pecking	Nest gathering	
Hopping		Nest building	

From Elaine Gurley-Fellars, "Non-Destructive Animal Study: Ringdoves, a Model Case" in *American Biology Teacher,* 43:420. Copyright © 1981 National Association of Biology Teachers, Inc., Reston, VA.

Table 31.8 Displays and Calls

Bow-cooing	A display given by adult males in sexual or aggressive encounters, and rarely seen in adult females. It may be seen in females under 5 months of age, but never in a mated female.
Nest calling	Seen in both sexes but more often in males. The bird takes on a posture of a 25-degree oblique angle with its head down and tail up in the vicinity of the future nest site. Cooing continues and the wings, held close to the body, are flicked. This call can be given on the nest, or floor or perch of the cage near the nest site. The female joins in this activity, which is sometimes known as "showing the nest site."
Ka-calling	The laughing call from which this species receives its name *"risoria"* means laughing. It is given in a number of contexts: as the bird arrives on its perch; as it dismounts its mate or rival; or as it chases a conspecific. Often, this call comes before a bow-coo, which is then followed by pecking or feather pulling of a conspecific. The female will ka-call during the male's initial courtship attempts.

Although rare in females, mounting is seen in both sexes. Other behavior activities seen are autopreening, allopreening, billing, pecking and feather pulling, wing boxing, driving and fleeing, and courtship feeding. Only the females perform crouching, usually just before copulation. She will crouch, lower her head, raise her shoulders, and move her tail to the side.

From Elaine Gurley-Fellars, "Non-Destructive Animal Study: Ringdoves, a Model Case" in *American Biology Teacher,* 43:420. Copyright © 1981 National Association of Biology Teachers, Inc., Reston, VA.

Reproductive Experiment with Ringdoves

After all laboratory sections have had an opportunity to make the preceding observations, male and female birds may be placed together in a cage permanently so that reproduction can occur. Courtship begins the first day. The male marches around the cage, bowing his head and cooing at the female. Later that day the pair will choose a place for their nest. Most likely, the only place in the cage will be the 10-cm nest bowl. Both birds will crouch in their nest site and utter a distinctive type of coo. The male will gather the nesting materials and pass them to the female, who constructs the nest while remaining in the bowl.

The female, as she vibrates her wing tips and tucks in the nest material, specifically stimulates the male to carry more nest material to the site. After a few days, the birds will copulate, and the female will become very attached to her nest. If she is determined to stay with the nest, when you try to lift her off it, she is very close to laying her eggs.

The first egg is usually laid about 5:00 P.M., between 7 and 11 days after courtship. Her second egg will be laid about 9:00 A.M. the next day. Then the pair takes turns sitting on the eggs. The male spends only 6 hours per day on the nest, compared to the 18 hours per day the female spends there. Body temperature in the male is higher and thus more beneficial to the eggs during the cooler portions of the night. Fourteen days later, the squabs hatch out of the eggs, and the parents begin to feed them crop milk. At 10–12 days old, the squabs can leave the nest, but they continue to beg food from their parents until they are 2 weeks old. As these young birds develop the pecking ability, the parents simultaneously lose interest in feeding them. Then the cycle begins again.

1. What behaviors of the male indicate that he invests energy in the reproductive process?
   a. prior to copulation (1) _____
      (2) _____
   b. after copulation (1) _____
      (2) _____
2. Might the precopulatory activities function to reassure a female that the male is willing to make a contribution to the reproductive process? _____
3. Experiments have shown that males will reject females who do not require a long courting display. Why is this a sound evolutionary practice for the male? _____
4. Male and female ringdoves contribute about equally to the reproductive process. Does this seem to correlate with their lack of sexual dimorphism (physical difference)? _____

Laboratory Review 31

_____ 1. Give an example of an innate behavior in humans.

_____ Give an example of learned behavior in humans.

_____ 2. What type of receptors seem to assist fruit flies and planarians in finding food?

_____ 3. Relate the complexity of the nervous system to the proportion of innate behavior and learned behavior of the organism.

4. Explain why animal behavior is dependent upon the anatomy and physiology of an organism.

5. What is the difference between a taxis and a reflex? Give an example of each in the organisms that you studied.

6. Why do birds sing more frequently in the spring than they do in the fall?

Reference

Brooklyn College of the City University of New York. 1973. *Biology 2.* Dubuque, IA: Kendall/Hunt Publishing Company.

Laboratory

32

Symbiotic Relationships

Learning Objectives

Students should be able to

1. define and give examples for each type of relationship studied;
2. identify all observed slides and plastomounts;
3. describe the life cycle of *Plasmodium,* tapeworm, blood fluke, and hookworm, and tell the manner in which these parasites can be acquired by humans.

Introduction

Symbiosis is a close relationship that may occur when 2 organisms of a different species live together. There are 3 types of symbiotic relationships, depending on which organisms benefit and to what extent they benefit. If both organisms benefit, it is referred to as *mutualism.* If one organism benefits and the other is unaffected, it is called *commensalism.* If one organism benefits while the other is harmed, it is called *parasitism.* Not all symbiotic relationships are as clear-cut as the definitions imply. For instance, commensalism may lean toward parasitism if disease, lack of food, or other circumstances change the balance.

Mutualism

Mutualistic relationships often help organisms obtain food or avoid predation.

Root Nodules of Leguminous Plants

The bacteria *Rhizobium* invade the roots of certain plants (clover, alfalfa, soybeans), where they form nodules (fig. 32.1). Here they obtain carbohydrates from their host and provide their host with organic nitrogen. They are a type of bacteria that is capable of *fixing nitrogen:* that is, changing aerial nitrogen to ammonia so that it can be incorporated into amino acids that are used by the plant to make its own proteins.

S 1. Study the living or preserved specimen of a plant with root nodules.

Does the root run through the center, or are the nodules to the side or at the ends of the root? _____
Figure 32.2 shows the manner in which the bacteria invade the plant and gives a cross section of cells within the resulting nodule.

S 2. Examine prepared slides showing cross sections of these cells. Note that they are filled with masses of stained bacteria.

Since bacteria are prokaryotic (if you do not remember the definition, look it up in the glossary of your text), should a membrane-bounded nucleus and multiple kinds of organelles be present? Why or why not?

_____

_____

Figure 32.1 Photograph of nodules on the roots of a legume.

Lichens

Lichens are composed of a *fungus* and an *alga* growing in intimate association. While the association is often considered *mutualistic,* it is likely that the fungus is somewhat parasitic on the alga.

S 1. Study figure 32.3 and note that fungi are composed of hyphae, while algae may be single cells.

S 2. Obtain a prepared slide of a lichen and note the *hyphae* and *algal cells* in close proximity within the thallus of the lichen. Your instructor may have on display different types of lichens (fig. 32.4): *crustose* (appressed to a substratum), *foliose* (leaflike lobes), or *fruticose* (erect or pendant branching structures).

S 3. Examine the lichens on display.

S 4. List the lichens on display by name and growth habit (e.g., crustose).

_____

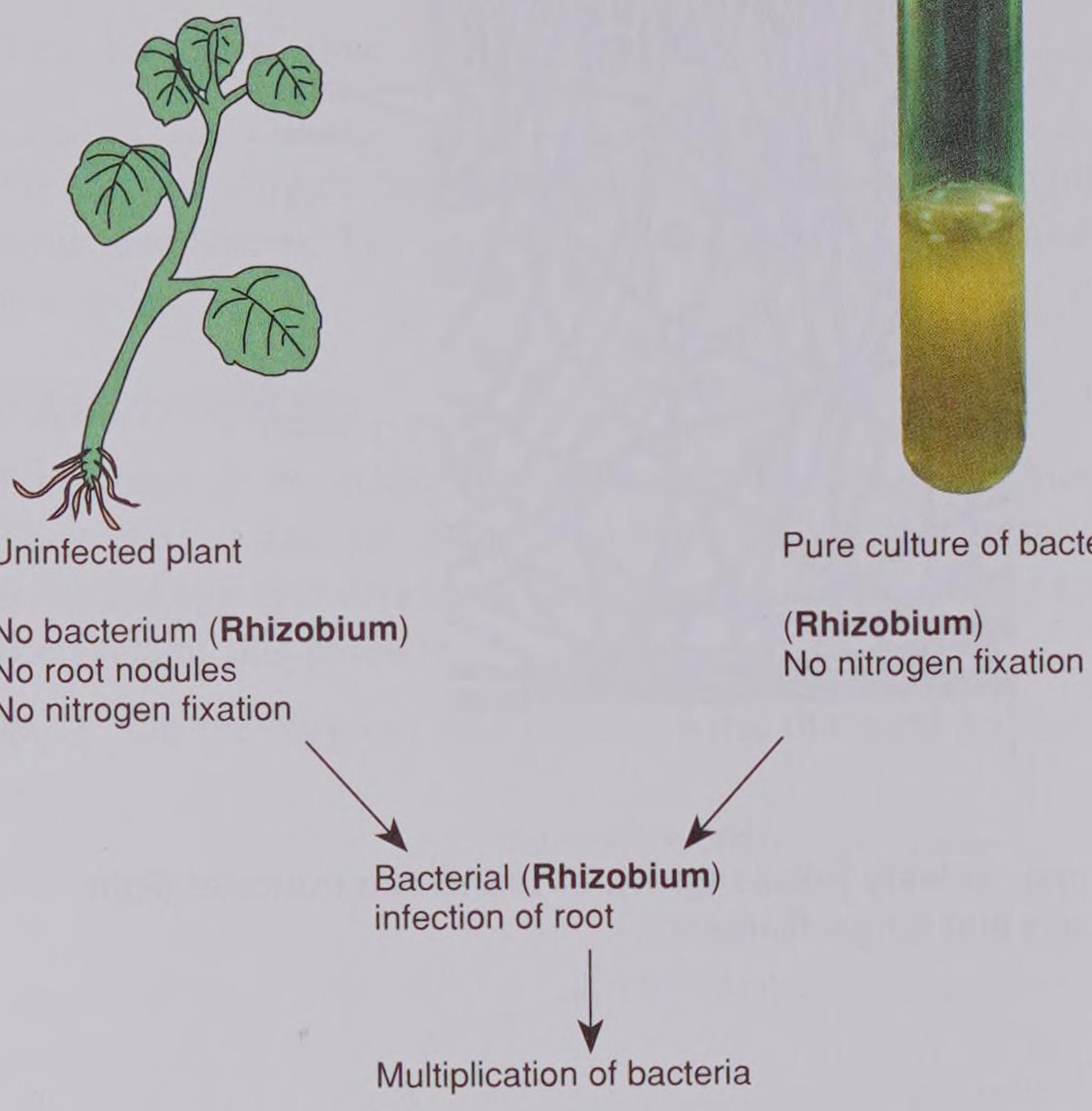
_____

A fruticose lichen called *reindeer moss* provides food for reindeer and caribou in the Arctic regions. Lichens are also important as soil-forming agents. Lichens can grow on rocks because the fungus sends acids into the rock and dissolves minerals while the alga carries on photosynthesis.

Which member of a lichen provides inorganic food? _____

Which member provides organic food? _____

Figure 32.2 Step in the infection of a legume (Pea family) plant by the symbiotic root nodule forming bacterium *Rhizobium*.

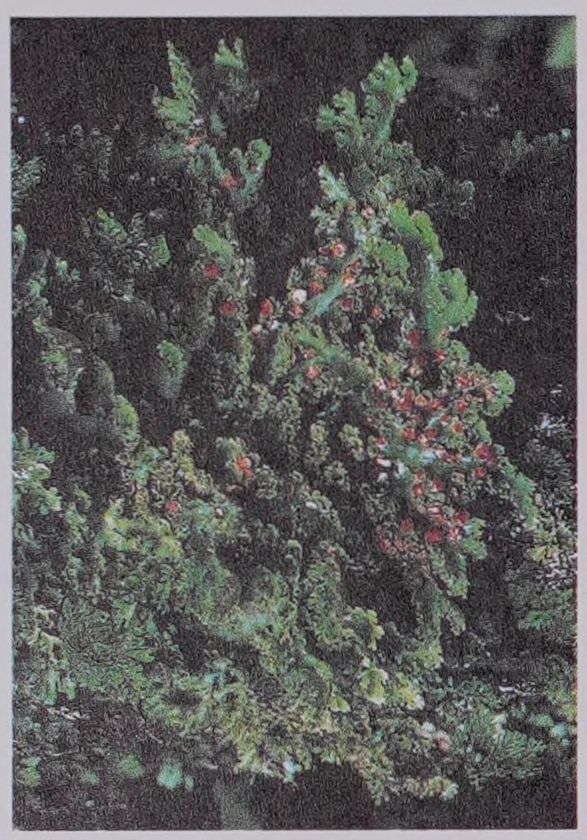


Termites

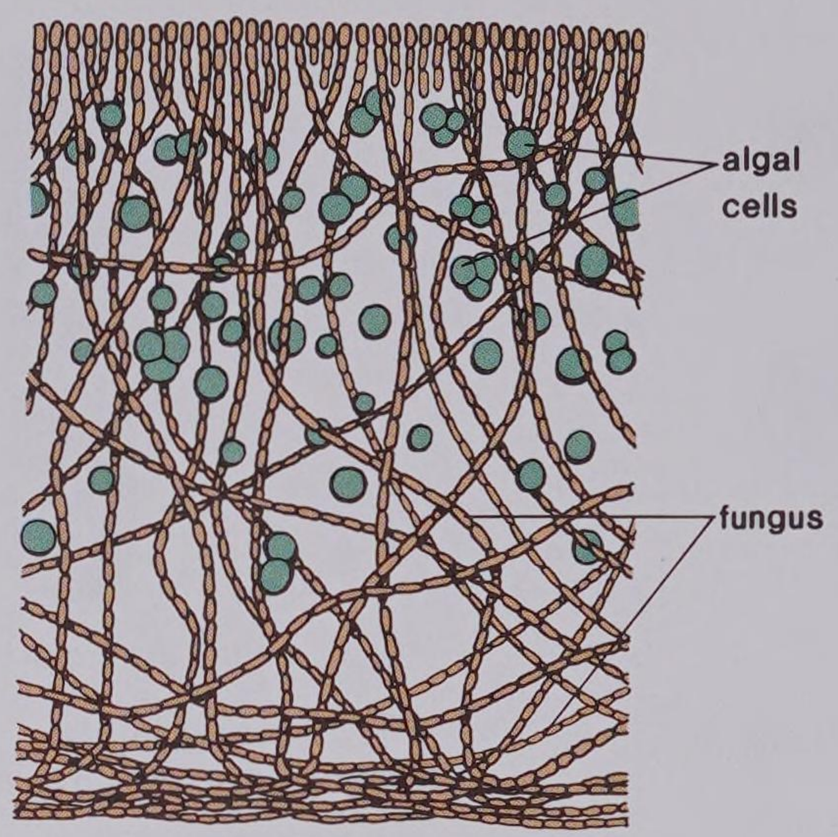
As is well known, *termites* have the capacity to eat wood. But when the matter is examined more closely, it is found that termites cannot digest wood. Instead, termites contain *symbiotic protozoans* (*Trichonympha* and *Pyrsonympha*), which do digest wood.

1. Place a drop of water on a clean slide.
2. Obtain a living wood-eating termite, if available.
3. Place it in the water on this slide with a dissecting needle.
4. Separate the abdomen from the head with a razor blade.
5. Tease the abdomen apart with a dissecting needle.
6. Cover with a coverslip and examine microscopically for moving creatures.
7. Carefully adjust the light source to enable you to see the characteristic shapes of the flagellates and, perhaps, their numerous flagella. If live specimens are not available, study a prepared slide of *Trichonympha* and *Pyrsonympha*.
8. Sketch the flagellated protozoan that you have isolated in the following space.

Figure 32.3 ***a.*** Photo of a lichen. ***b.*** Diagram illustrating internal structure.

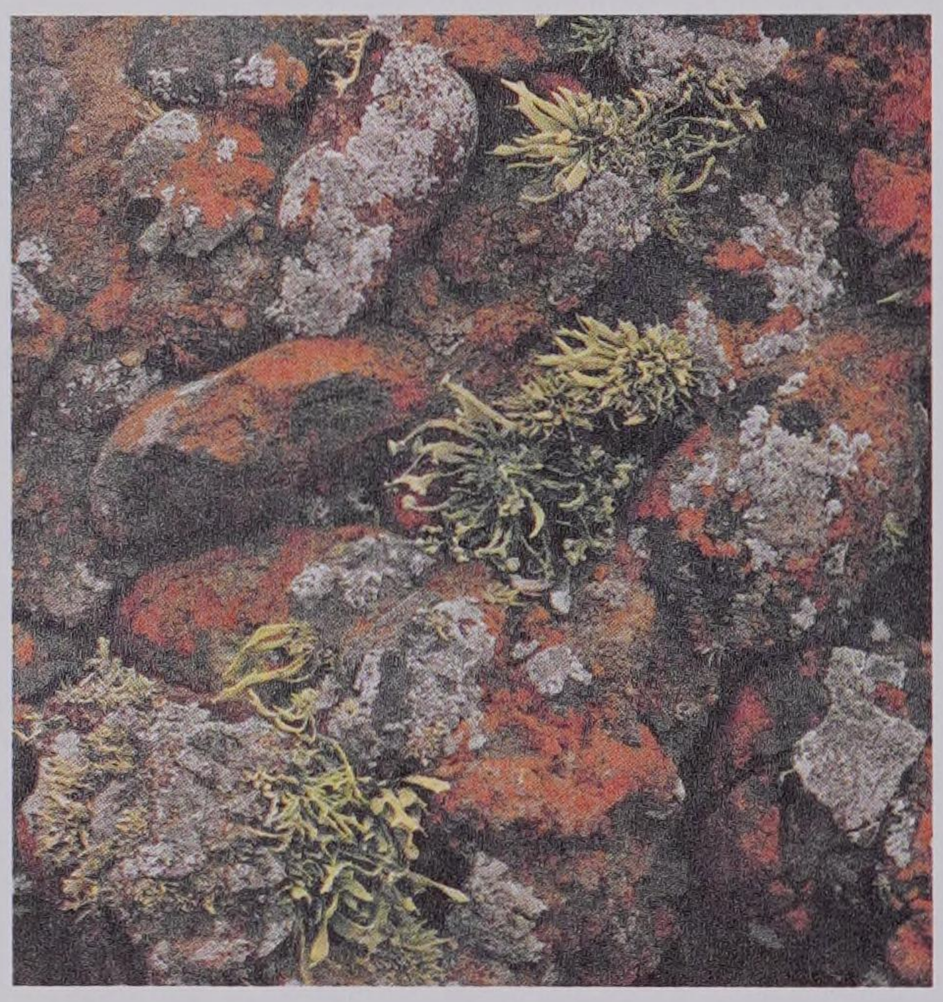


a.



b.

Figure 32.4 Lichens can be either low lying crustose (orange), or leafy foliose (grey), or branchlike fruticose (light green). All types are a symbiotic relationship between algal cells and fungal filaments.



Commensalism

In a commensal relationship between 2 species, only one of the species benefits; the other is neither benefited nor harmed. Often, the latter provides a home and/or transportation for the former.

Entamoeba gingivalis

- S 1. Put a drop of *0.9% NaCl* solution on a glass slide.
- S 2. Gently insert a (prepackaged) toothpick into the gum pockets at the base of your teeth and remove some material.
- S 3. Deposit this material in the drop on the glass slide by swirling the pick in the liquid.
- S 4. Cover with a coverslip.
- S 5. Examine the slide using the low-power objective and rather weak illumination.
- S 6. Look for rather large cells that are motile and have pseudopodia.

These organisms are found more frequently in persons with gum disease, although it is not clear whether they bring on or aggravate the condition.

Parasitism

A parasite derives nourishment from a host and usually does the host some harm. It is better for the parasite, however, if the host stays alive. Why? _____

_____

Viruses are *obligate parasites* (reproducing only inside living cells); there are also parasites among *bacteria, protozoans, fungi,* and *worms*. The latter are often severely modified and adapted to their way of life, and they may have complicated life cycles.

Plasmodium

Protozoans of the genus *Plasmodium* cause malaria in human beings. These protozoans have no means of locomotion. They exist as asexual spores that repeatedly divide mitotically or as sex cells; these sex cells join together to form a fertilized egg that divides to produce cells that begin the spore-producing process. Study figure 32.5, which depicts the life cycle of this parasite.

S 1. On the diagram that follows, write the word *human* and the word *mosquito* where appropriate.

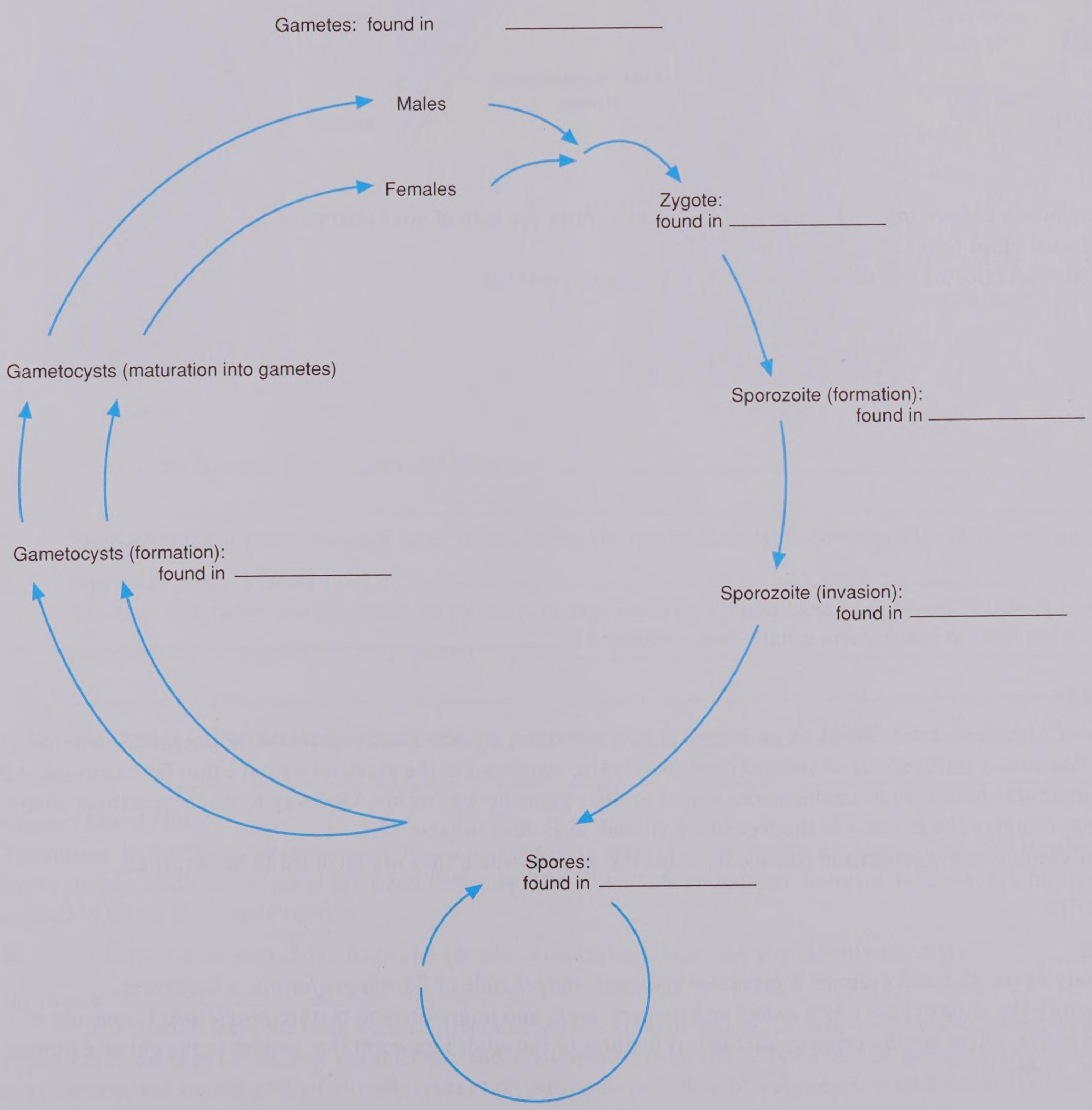
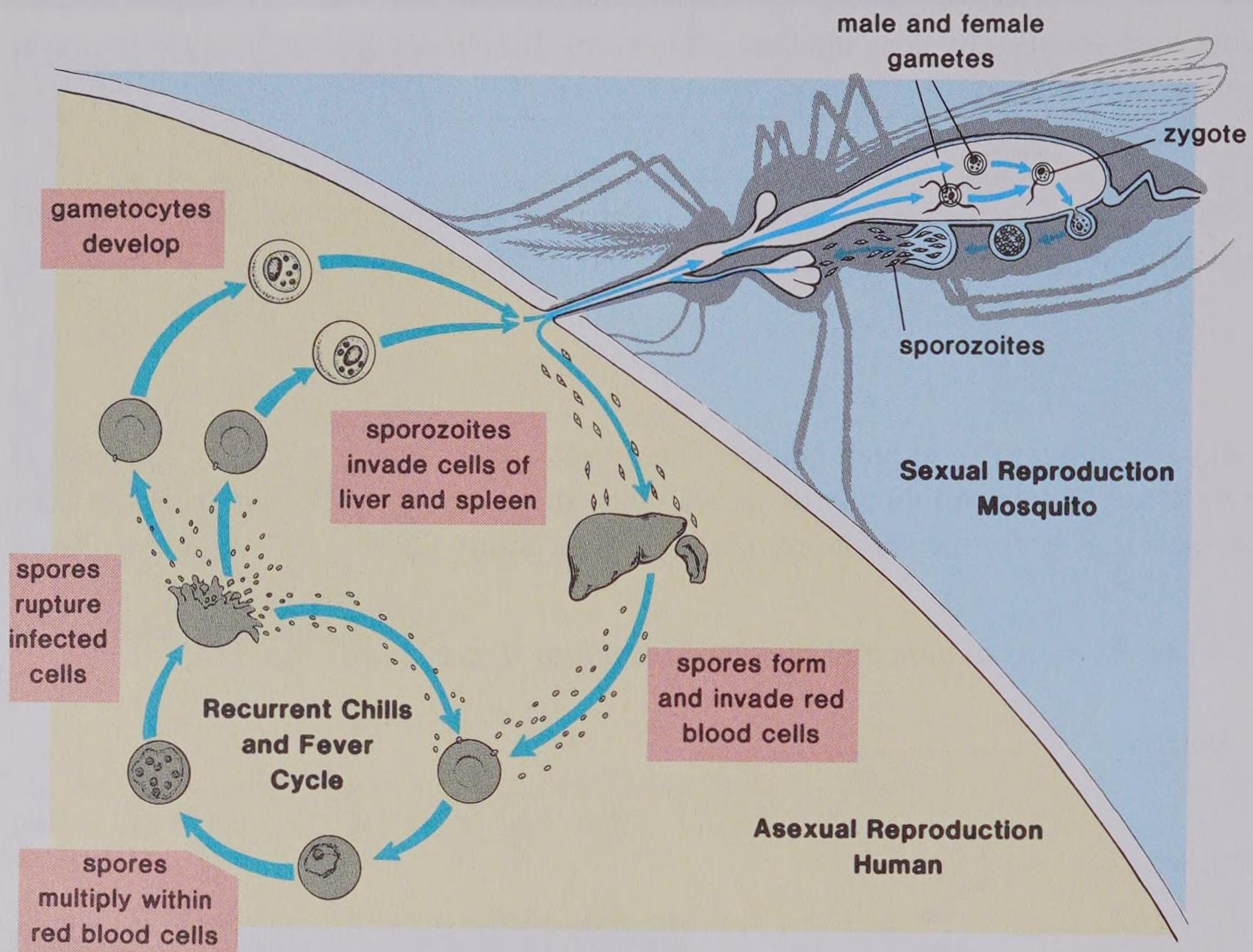


Figure 32.5 Life cycle of *Plasmodium,* a protozoan that causes malaria.



Ⓢ 2. Examine a slide of infected human red blood cells. With the help of your instructor, locate and identify the infected blood cells.

Ⓢ 3. Sketch an infected and normal blood cell in the space provided.

By what method has malaria usually been controlled? _____

Worms

A variety of organisms are referred to as worms (e.g., tapeworms, roundworms, segmented worms), and some infect humans. Worms are multicellular animals of considerable size compared to the parasites we have thus far examined. All of these organisms have various modifications suited to their parasitic way of life. One way to emphasize these modifications is to compare the parasite to the free-living animal, as is done in table 32.1.

Ⓢ As you examine the organisms in this section, note the ways in which they are modified to be parasites.

Flatworms

Tapeworm

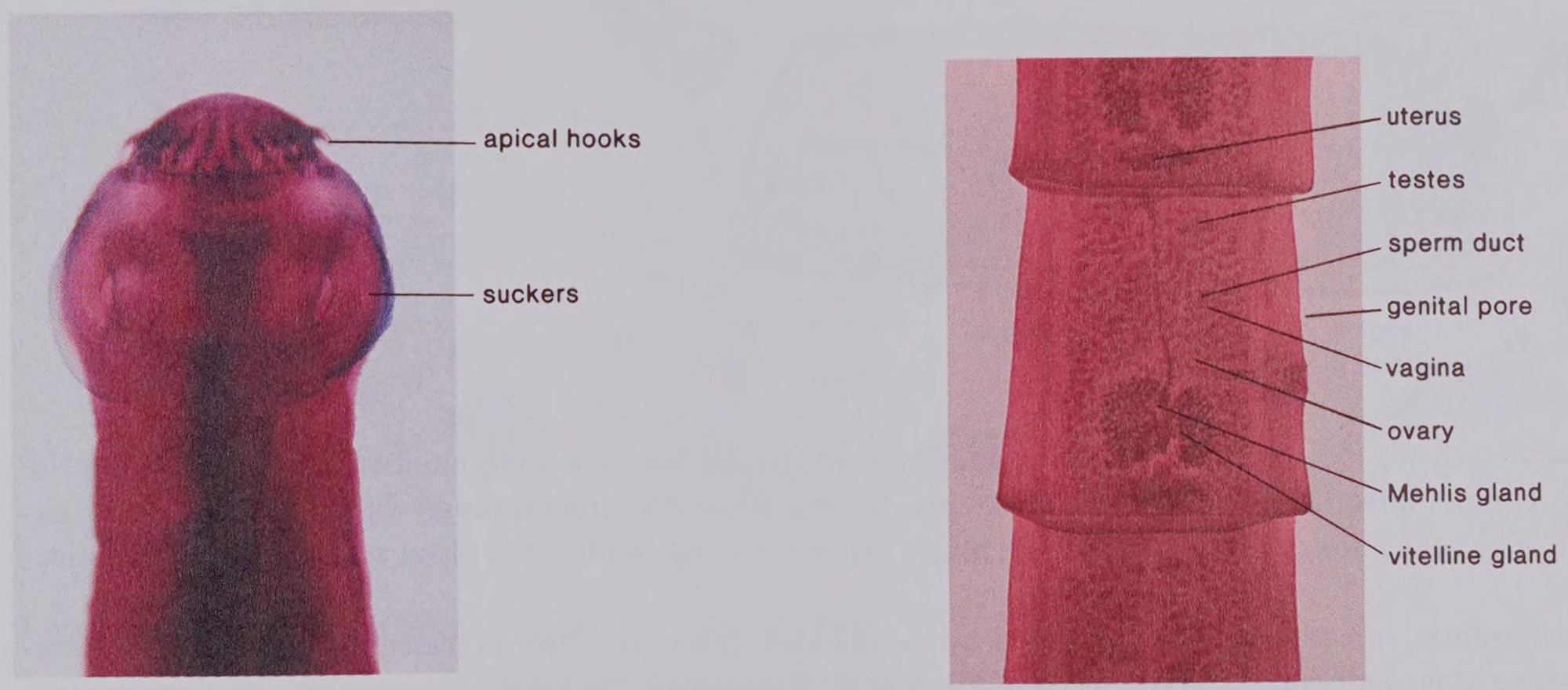
Ⓢ 1. Study figure 32.5 and examine a preserved specimen and/or slide of *Taenia pisiformis,* a tapeworm.

Ⓢ 2. Identify the *scolex* (head) with *hooks* and *suckers, neck,* and immature and mature *proglottids* (segments of the body). These are the primary anatomical features of the adult tapeworm that resides in the gut of a human (fig. 32.6).

Table 32.1	Free-Living Worm Compared to Parasite

Free-Living	Parasite
1. Well-developed nervous system	1. Reduced nervous system
2. Sense organs such as eyes	2. Sense organs such as those of touch
3. Fast moving, with protective devices	3. Locomotion limited
4. Well-developed muscles	4. Minimal muscle fibers
5. Efficient circulatory system	5. Reduced circulatory system
6. Normal reproduction	6. Complicated life cycle

Figure 32.6 Tapeworm anatomy. ***a.*** Scolex. ***b.*** Proglottid.



a. Scolex b. Proglottid

What is the function of the hooks and suckers? _____

_____

Proglottids mature into "bags of eggs." Considering the life cycle of the tapeworm (fig. 32.7), why might a tapeworm produce so many eggs? _____

The eggs of a tapeworm are taken up by cattle or pigs and develop into larvae (embryos) that encyst in the muscle. How do humans get a tapeworm? _____

_____

What might be a protection against getting a tapeworm? _____

_____

Human Blood Fluke

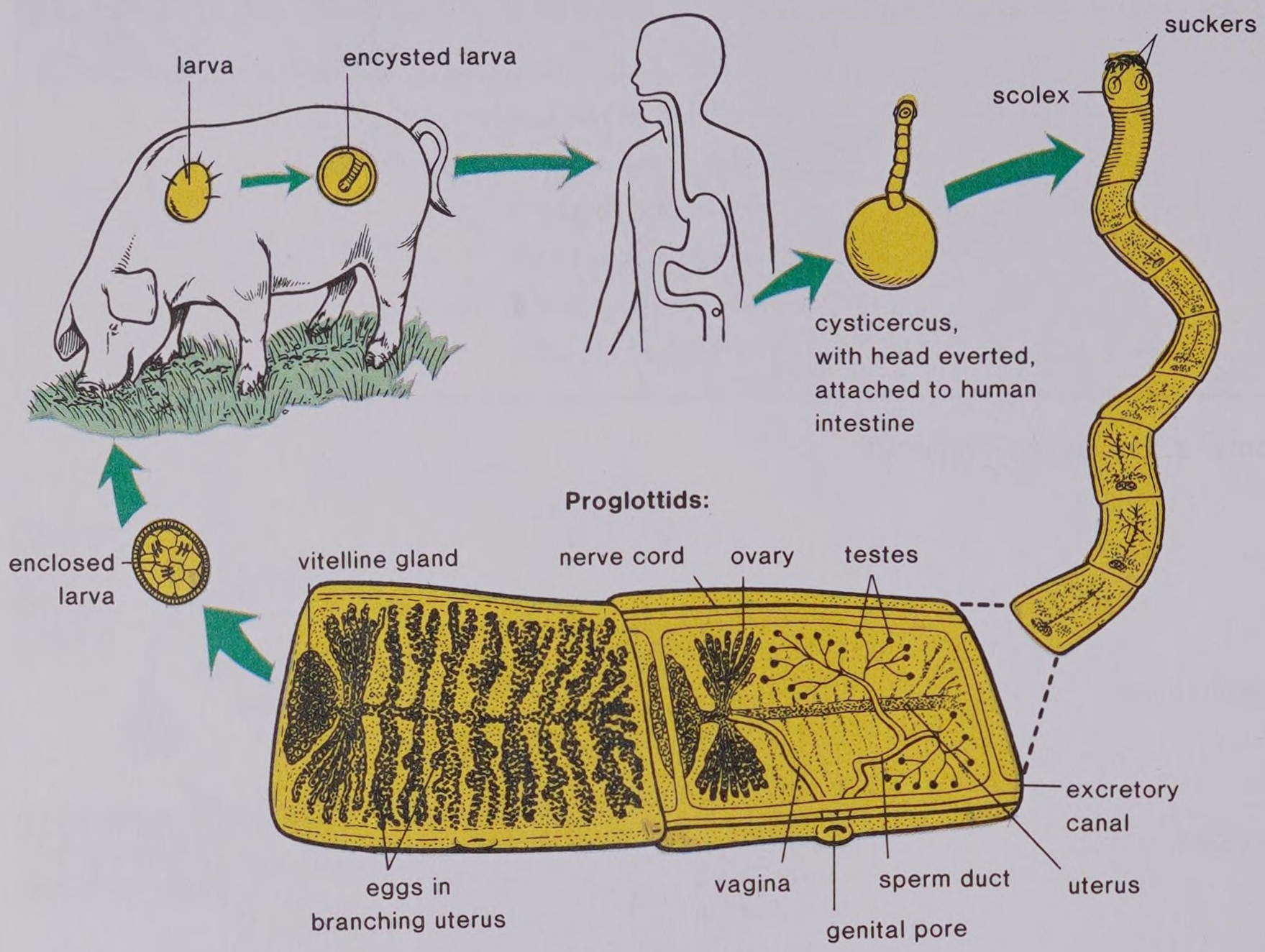
The human blood fluke, *Schistosoma mansoni,* has separate sexes. Female worms are round, long, and slender (1.2–2.6 cm in length), while males are shorter and flattened. The males look cylindrical, however, because they incurve to form a canal in which the female resides.

Obtain a slide that shows male and female in copulation. How can you identify the male? _____

the female? _____

The females leave the male worms to deposit their ova in small venules, close to the intestinal passageway (lumen). From there, the eggs escape into the intestine and are liberated from the body by way of the feces. In fresh water the eggs rupture, and the miracidia (ciliated larvae) that escape swim in search of appropriate snail hosts. If successful, they

Figure 32.7 Life cycle of tapeworm (*Taenia*), showing mature proglottid (*right*) and grand proglottid (*left*) in detail.



penetrate the snails; there, they undergo a cycle of development, giving rise to a large number of cercariae (tadpole-shaped larvae). Human infection takes place by direct penetration of the cercariae through the skin to invade the circulatory system. The young flukes mature in the liver; as adults, they take up residence in blood vessels near the intestines (fig. 32.8).

Schistosomiasis, infection of humans caused by blood flukes, is an extremely prevalent disease in Egypt, especially since the time of the building of the Aswan dam. The standing water of irrigation ditches, combined with unsanitary practices, has created the conditions necessary for widespread infection.

There are other types of flukes that affect different organs of the body.

Ⓢ Observe the larger liver or lung flukes on display. List their names.

_____

_____

Roundworms

Most roundworms are free-living, but a few are parasitic.

Ⓢ 1. To gain an overall impression of the anatomy of a roundworm, remove a small sample from a culture of *Rhabditis.*

Ⓢ 2. Place it in a drop of water on a slide. Cover with a coverslip and examine. If the culture is not too old, there will be many eggs and larvae, as well as adult males and females.

3. Describe the characteristic movement of roundworms. _____

_____

Trichinella

Trichinella are roundworms that cause the disease *trichinosis.*

Ⓢ Examine preserved, infected meat or a slide of infected meat and locate the cysts, which contain the juvenile worms. When humans eat infected meat, the juvenile worms are released in the digestive tract and mature to reproduce sexually, again producing juvenile worms that infect human muscle (fig. 32.9). A human with trichinosis has muscular aches and pains that can lead to death if respiratory muscles fail. Trichinosis can be prevented by thorough cooking of all pork and pork products.

Figure 32.8 Life cycle of blood fluke (*Schistosoma*).

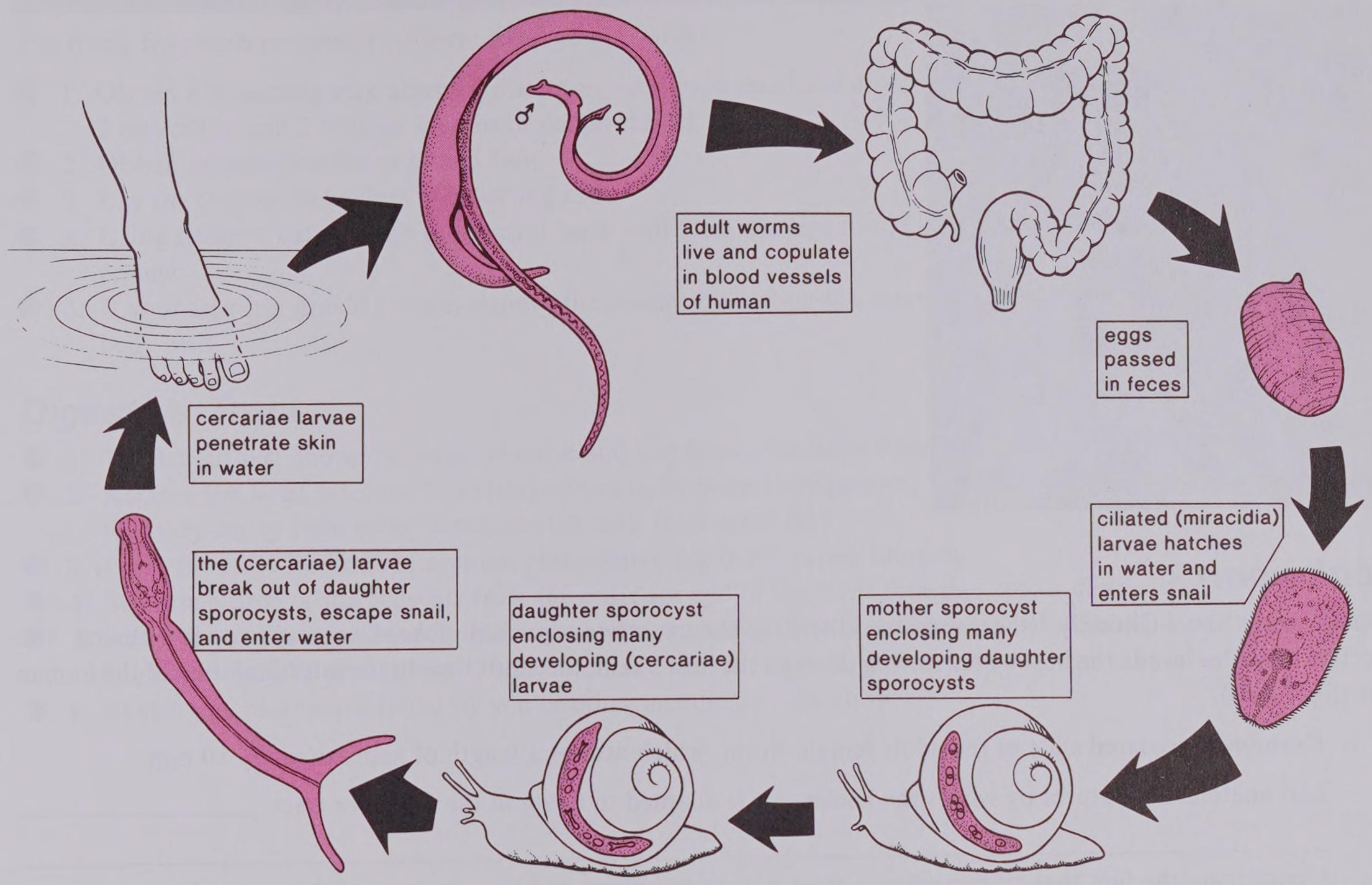

♂
♀
adult worms live and copulate in blood vessels of human
eggs passed in feces
ciliated (miracidia) larvae hatches in water and enters snail
mother sporocyst enclosing many developing daughter sporocysts
daughter sporocyst enclosing many developing (cercariae) larvae
the (cercariae) larvae break out of daughter sporocysts, escape snail, and enter water
cercariae larvae penetrate skin in water

Figure 32.9 *Trichinella* larva embedded in a muscle fiber.

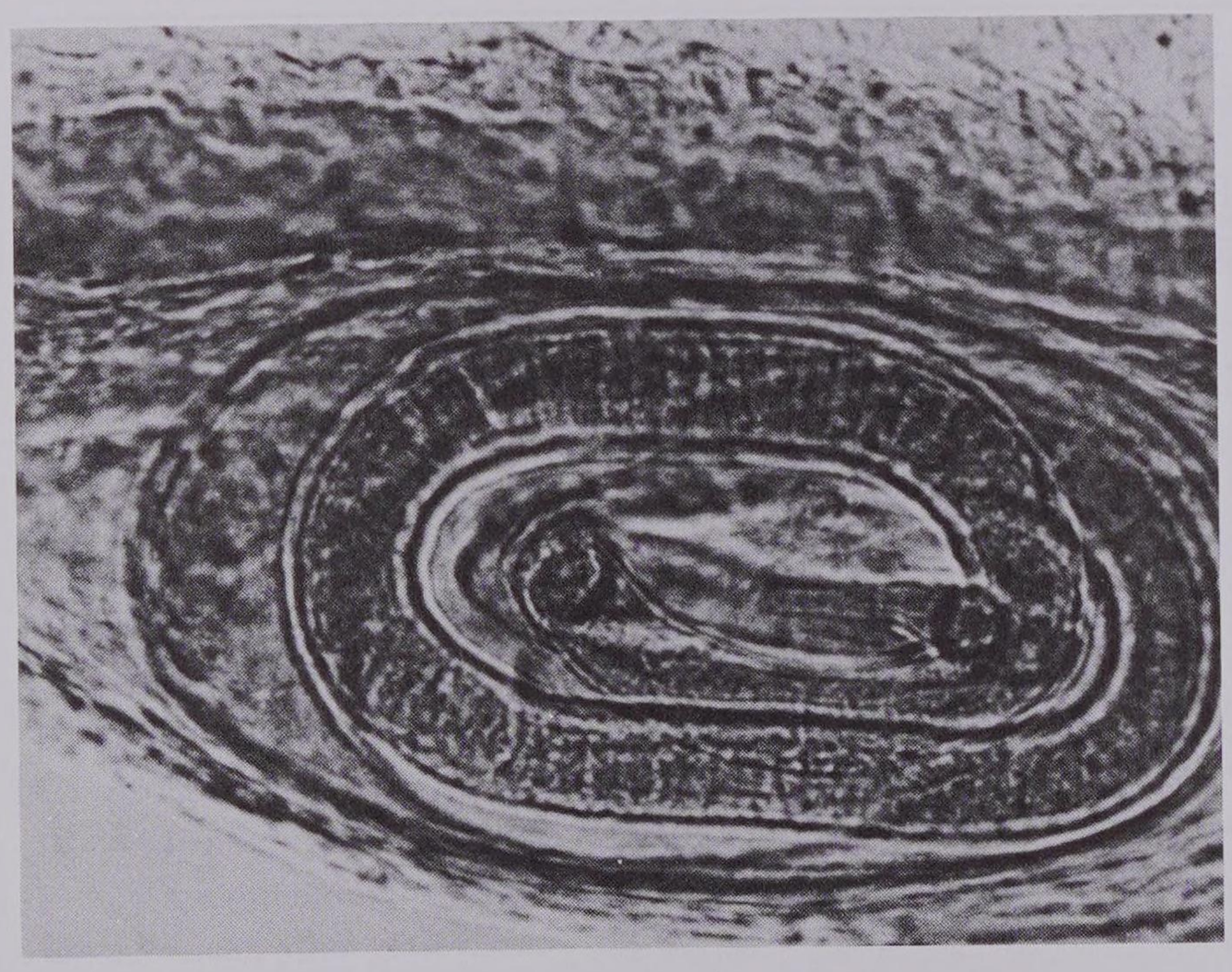


Figure 32.10 Longitudinal section through hookworm attached to intestinal wall.

Hookworm

Necator americanus (literally, "the American killer") is the causative organism of *hookworm disease* in humans. The infective juveniles invade the host by burrowing through the host's skin; the adult lives in the intestinal tract of the human host (fig. 32.10).

S 1. Examine a prepared slide of the adult female worm, which attains a length of approximately 10 mm.

List anatomical features by which the hookworm is adapted to living in the digestive tract. _____

_____

Considering the fact that transmission is from human to human and that the eggs of these organisms pass out with the feces, how can we best prevent transmission? _____

_____

S 2. Examine any additional worms on display and the ways they are modified to be parasites.

S 3. List their names and the common name of the category (e.g., roundworm, flatworm) to which they belong in the space provided.

_____

_____

S 4. Select one of these worms, write its name, and sketch it in the following space.

Frog Dissection (Optional)

The living frog harbors several different kinds of parasites.

1. Obtain a dissecting tray, scissors, dissecting needles, a medicine dropper, a clean watch glass, 2 clean slides and 2 coverslips, and 2 12-inch lengths of cotton thread.
2. Obtain an anesthetized or pithed frog.
3. Lay the frog on its back in a dissecting pan.
4. Using scissors, cut through the ventral body wall from the legs to the jaw in order to expose the internal organs.
5. If your frog is a gravid female, remove the ovaries by cutting the membranes that attach them to the dorsal body wall.

Digestive Tract

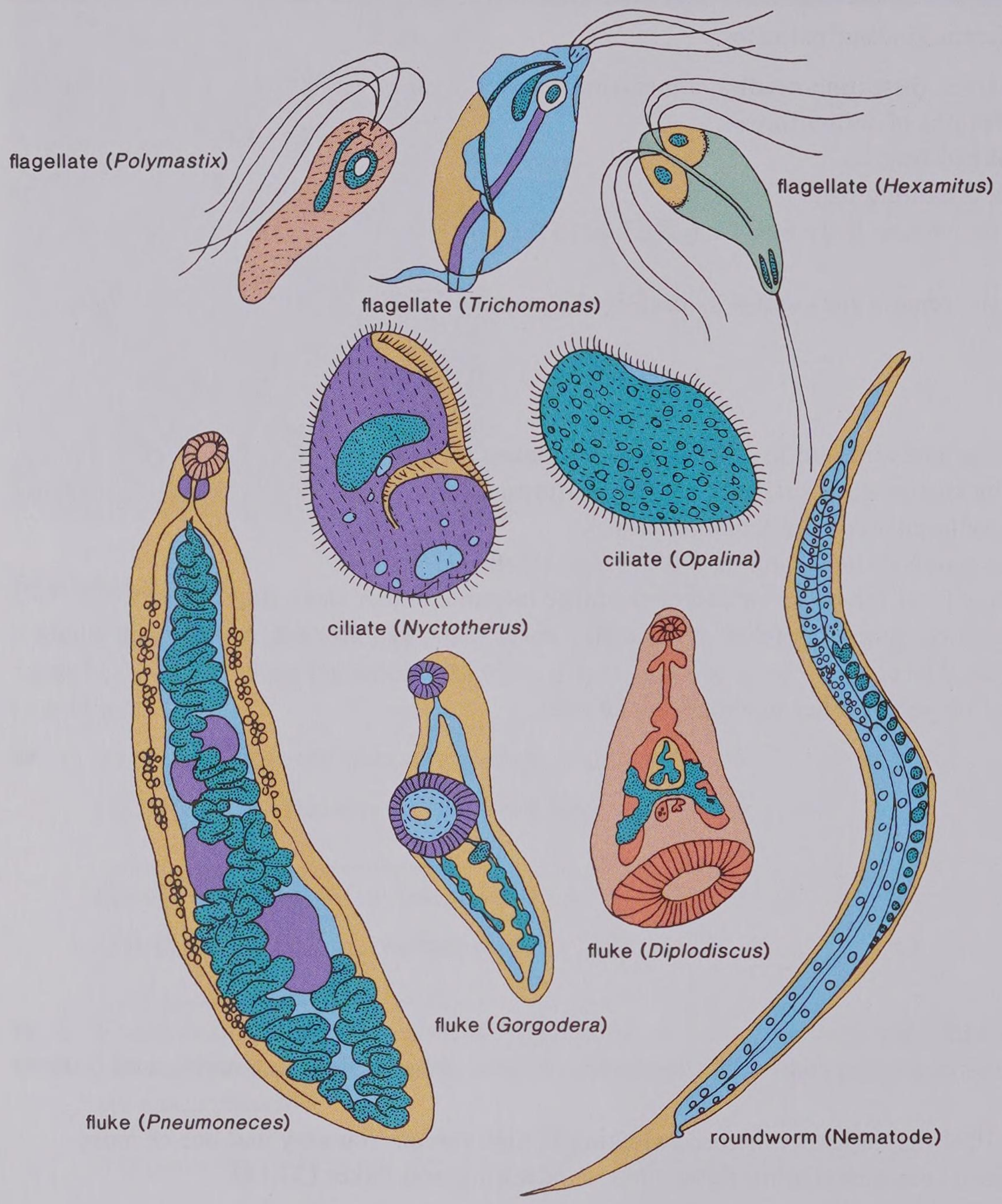
1. Tie the gut just above the large intestine and just below the large intestine.
2. Remove the large intestine by cutting across it, between the ligatures, at both ends. (This method will prevent the body cavity from being contaminated with fecal material.)
3. Place the large intestine in a watch glass containing *0.9% saline* solution.
4. Squeeze a drop of the contents from the posterior end of the large intestine onto a clean slide.
5. Add a few drops of *saline* solution, mix thoroughly, cover with a cover glass, and observe. A protozoan ciliate (*Opalina*) may be present (fig. 32.11).
6. Sketch any protozoans found by you or other members of the class.

7. Slit open the large intestine with your scissors.
8. Transfer a drop of the contents to a clean slide, add a few drops of *saline* solution, mix thoroughly, and cover with a coverslip.
9. Observe with both 4× and 10× magnification before switching to high power. You may find one or more species of unicellular organisms, occasional adult flatworms, roundworms, and flukes (32.11).
10. Open and examine the rest of the digestive tract in place.
    The small intestine sometimes contains roundworms of various kinds and sizes, but the stomach rarely contains parasites. Why? _____
11. Clean your glassware.

Bladder

1. Remove the urinary bladder.
2. Transfer it to a watch glass containing *saline* solution.
3. Spread the elastic tissues with dissecting needles and look carefully at every part.
4. If anything moves, carefully detach it and mount it for microscopic examination with 4× and 10× magnification. Flukes are commonly found (fig. 32.11).
5. Clean your glassware.

Figure 32.11 Common parasites found in frog internal organs.



Lungs

- 1. Remove the lungs and put them in a watch glass containing *saline* solution.
- 2. Tease the lung tissue apart with 2 dissecting needles. If anything moves, carefully detach and mount the moving object for microscopic examination with 4× and 10× magnification. Flukes and roundworms are commonly found (fig. 32.11).
- 3. For each intestinal organism found by you, or the members of your class, list the names and the common names of the category (e.g., roundworm, flatworm, protozoan) to which each belong. _____

_____

_____

Laboratory Review 32

_____ 1. What type of organism causes malaria?

_____ 2. What type of reproduction produces spores?

_____ 3. What name is given to the segments of a tapeworm's body?

_____ 4. Would you expect to find eyes in a parasitic or free-living organism? Why?

_____ 5. What type of organism causes schistosomiasis?

_____ 6. What organism is the secondary host for malaria?

_____ 7. Do parasites expend more energy on maintenance or on reproduction?

8. What is the difference between parasitism and mutualism?

Reference

Andresen et al. 1980. *Laboratory Inquiries into Concepts of Biology.* Dubuque, IA: Kendall/Hunt Publishing Company.

Laboratory

33

Analysis of an Ecosystem

Learning Objectives

Students should be able to

1. define all the terms used in the laboratory instructions;
2. describe the interrelationship between organisms and their environment.

Introduction

Ecology is unique as a biological discipline because it is the only science that deals with the organism at the supraorganismal level (fig. 33.1). The word *ecology* is derived from the Greek, meaning "house" or "place to live." Ecology is the study of groups of organisms (populations) functioning in the environment (ecosystem).

Any ecosystem consists of *abiotic* (nonliving) and *biotic* (living) components and the energy flow through the system. The abiotic components are the basic *inorganic* and *organic* compounds and other *nonliving* factors such as weather.

The biotic component is comprised of (1) *producers,* or autotrophic organisms, (2) *consumers,* or heterotrophic organisms, and (3) *decomposers,* or saprophytic heterotrophs. In this laboratory, you are asked to examine an ecosystem and identify as many components as possible. To aid you in identifying biotic organisms, simple keys to the plant and animal kingdoms are provided.

Figure 33.1 Levels of biology. (From Paul, C., 1975. *Principles of Biological Science.* Dubuque, IA: Kendall/Hunt Publishing Company.)

PROTOPLASM → CELLS → TISSUES → ORGANS → ORGAN SYSTEM → ORGANISMS → POPULATIONS → COMMUNITIES → ECOSYSTEMS → BIOMES → BIOSPHERE

realm of other biological science, i.e., genetics, development

realm of ecology (supraorganismal level)

Terrestrial Ecosystem

The ecosystem to be examined may be a 1 square meter or larger of marine environment, a forest, or 1 square meter of a field as chosen by your instructor.

- Take with you the equipment you need, such as meter sticks, collecting jars, nets, pH paper, and thermometer.

Procedure in the Field

1. Measure off the area to be examined with a meter stick.
2. Draw in the space provided a 3-dimensional *plot plan.*
3. Indicate on the plan any features in the abiotic environment, such as *soil, soil surface, air,* or any others that you find significant.
4. Sketch a *plot plan* of your terrestrial ecosystem.
5. Describe on a separate paper any supplemental information about your ecosystem.
6. Number each collection site on your plot plan.
7. Record this number in parentheses by each set of data that is recorded in tables 33.1–33.2.
8. Measure the current abiotic factors: *air temperature, wind velocity, wind direction, soil temperature,* and other factors as directed by your instructor.
9. Record these measurements in table 33.1.
10. Collect samples of organisms. These may be found *beneath* the surface, on the *surface,* or in the *air.*
11. Take the temperature at *eye* level, *ground* level, and *beneath* ground level.
12. Check the pH of the soil or water with pH paper. Check in several spots and at different depths.
13. Observe topographical landmarks; note *coloration, soil type,* and any other significant data. Record on your plot plan. Take several soil samples back to the laboratory.
14. Determine whether your biotic samples are common to several areas of the ecosystem. Record your samples—on the collection jar and later on your data sheet—as *rare, occasional,* or *abundant.* Many organisms can be studied alive in the field and released or examined in the laboratory and returned to the same environment soon.
15. (Optional) Expose sterile petri dishes containing bacterial medium to determine the presence of bacteria and fungi.

Procedure in the Laboratory

1. Key out any organisms by examining them under the binocular dissecting microscope and then using the keys provided by your instructor. Note whether they are *producers, consumers,* or *decomposers* and record them on the data sheet.
2. (Optional) Inoculate tubes of sterile medium with water and soil samples. Incubate and later inoculate plates to determine the presence of decomposers in your samples.

Table 33.1 Abiotic Factors—Terrestrial Ecosystem Analysis

Date: _____	Time: _____
Abiotic Factor	**Measurement (include units)**
Air temperature: Eye level	
Ground level	
Wind velocity	
Wind direction	
Soil temperature (beneath ground level)	
(Enter any additional factors in the spaces below)	

Table 33.2 Biotic Factors—Terrestrial Ecosystem Analysis

Type of Ecosystem _____

Constituents	Location in Ecosystem	Abundance Rare Occasional Abundant	Classification (phylum/class)	Identifying Feature for Classification
Producers				
Consumers				
Decomposers				

3. Select any 4 producers and any 4 consumers from the organisms collected, and explain how they are adapted to the terrestrial environment in which they occur.

Organism:	**Adaptation:**
Producers:	
_____	_____
_____	_____
_____	_____
_____	_____
Consumers:	
_____	_____
_____	_____
_____	_____

Aquatic Ecosystem Analysis (Optional)

If an aquatic ecosystem is available for study, follow the directions given.

1. Measure the current abiotic factors: *water temperature* at the surface and any other depths for which you have equipment to measure; *pH; turbidity;* dissolved *oxygen;* chemical nutrients, *nitrates, phosphates,* and other chemicals for which you have equipment; and any other abiotic factor(s) for which you have equipment.
2. Enter data in table 33.3.
3. Measure depths and observe currents. Record on the *plot plan,* and take several water samples back to the laboratory.
4. Number each collection site on your plot plan.
5. Record this number in parentheses by each set of data that is recorded in tables 33.3–33.4.
6. Sketch a plot plan of your pond, stream, or other aquatic ecosystem.
7. Describe on a separate paper any supplemental information about your ecosystem.
8. Collect samples of organisms. Many organisms can be studied alive in the field and released or examined in the laboratory and returned to the same environment.
9. Key out any organisms by examining them under the binocular dissecting microscope and then using the keys provided by your instructor.
10. Note whether they are *producers, consumers,* or *decomposers* and record them on the *data sheet.*
11. Make wet mounts of each water sample.
12. Examine these samples with the compound light microscope.
13. Note any living organisms and record them on the data sheet as *producers, consumers,* or *decomposers.*

Table 33.3 Abiotic Factor Measurements—Aquatic Ecosystem Analysis

Date: _____	Time: _____
Abiotic Factor	**Measurement (include units)**
Water temperature Surface ( )	
pH	
Turbidity	
Dissolve oxygen	
Nutrients: Nitrate Phosphates ( ) ( )	
(other)	

Table 33.4 Biotic Factors—Aquatic Ecosystem Analysis
Type of Ecosystem

Constituents	Location in Ecosystem	Abundance Rare Occasional Abundant	Classification (phylum/ class)	Identifying Feature for Classification
Producers				
Consumers				
Decomposers				

12. Select any 4 producers and any 4 consumers from the organisms collected, and explain how they are adapted to the aquatic environment in which they occur.

Organism:	**Adaptation:**
Producer:	
_____	_____
_____	_____
_____	_____
_____	_____
Consumers:	
_____	_____
_____	_____
_____	_____

Laboratory Review 33

Define the following terms:

1. Ecosystem: _____

   _____

2. Abiotic: _____

   Example: _____

3. Biotic: _____

   Example: _____

4. Producers: _____

   Example: _____

5. Consumers: _____

   Example: _____

6. Decomposers: _____

   Example: _____

Reference

Paul, C. 1972. *Principles of Biological Science.* Dubuque, IA: Kendall/Hunt Publishing Company.

Laboratory

34

Effects of Pollution

Learning Objectives

Students should be able to

1. state the effects of the various test solutions on the growth of lower organisms, seeds, and *Gammarus;*
2. use the results of today's experiments to predict the effect of pollutants on natural ecosystems;
3. define cultural eutrophication and relate this concept to the integrity of ecosystems.

Introduction

In an ecosystem, populations of organisms interact among themselves and the physical environment. Figure 34.1 shows that the size of each trophic feeding level within the community (all the populations taken together) is appropriate to maintain the ecosystem. The increase or decrease of any one trophic level can seriously threaten the entire ecosystem.

Today we will study the effect of various pollutants on organisms and hypothesize how these pollutants might affect the integrity of ecosystems. To simulate the effects of *acid rain,* we will test the effect of acid pH on the behavior and survival of organisms; to simulate the effects of *chemical pollution,* we will test the effect of 2 concentrations of pesticides on the behavior and/or survival of organisms; and to simulate the effects of *thermal pollution,* we will test the effect of water boiled and kept at 31° C. Boiling drives dissolved oxygen from the water.

The integrity of ecosystems can also be upset by the addition of excess nutrients to the system. With time, bodies of water naturally undergo an enrichment process called *eutrophication.* Eutrophication leads to overgrowth that can even cause an eventual filling in and disappearance of a pond or lake. Sometimes human activities add an excess of nutrients to bodies of water, and this occurrence is termed *cultural eutrophication.* Today we will also consider this ecological problem.

Hay Infusion

Hay infusion cultures contain various microscopic organisms such as those depicted in figure 34.2. Examine each of the cultures available by preparing a wet mount. The control culture best simulates the conditions that are optimum for normal growth.

In this lab, you will test the effects of chemicals on invertebrate organisms. These chemicals are in concentrations that will not harm you when handled properly. Please observe all *precautions* as instructed. Place used pesticide and pH-adjusted solutions in the correct *disposal containers*. *Wash* your hands well at the end of the laboratory period.

1. *Control culture*—sterile spring water, hay, or wheat inoculated with invertebrate organism(s).
2. *Enriched medium*—same as control but with more inorganic nutrients for the growth of photosynthetic organisms.
3. *Lack of oxygen*—same as control, but minimal oxygen. Culture was grown with minimal O_2 to test the effect of anaerobic conditions on the organism. *Anaerobic* conditions sometimes develop in nature when organic matter (e.g., sewage) is added to a body of water. The presence of organic matter causes the bacteria of decay to increase in number. The increased use of oxygen caused by the bacteria as they consume the organic matter results in anaerobic conditions.

Figure 34.1 A pyramid of energy of a Minnesota lake.

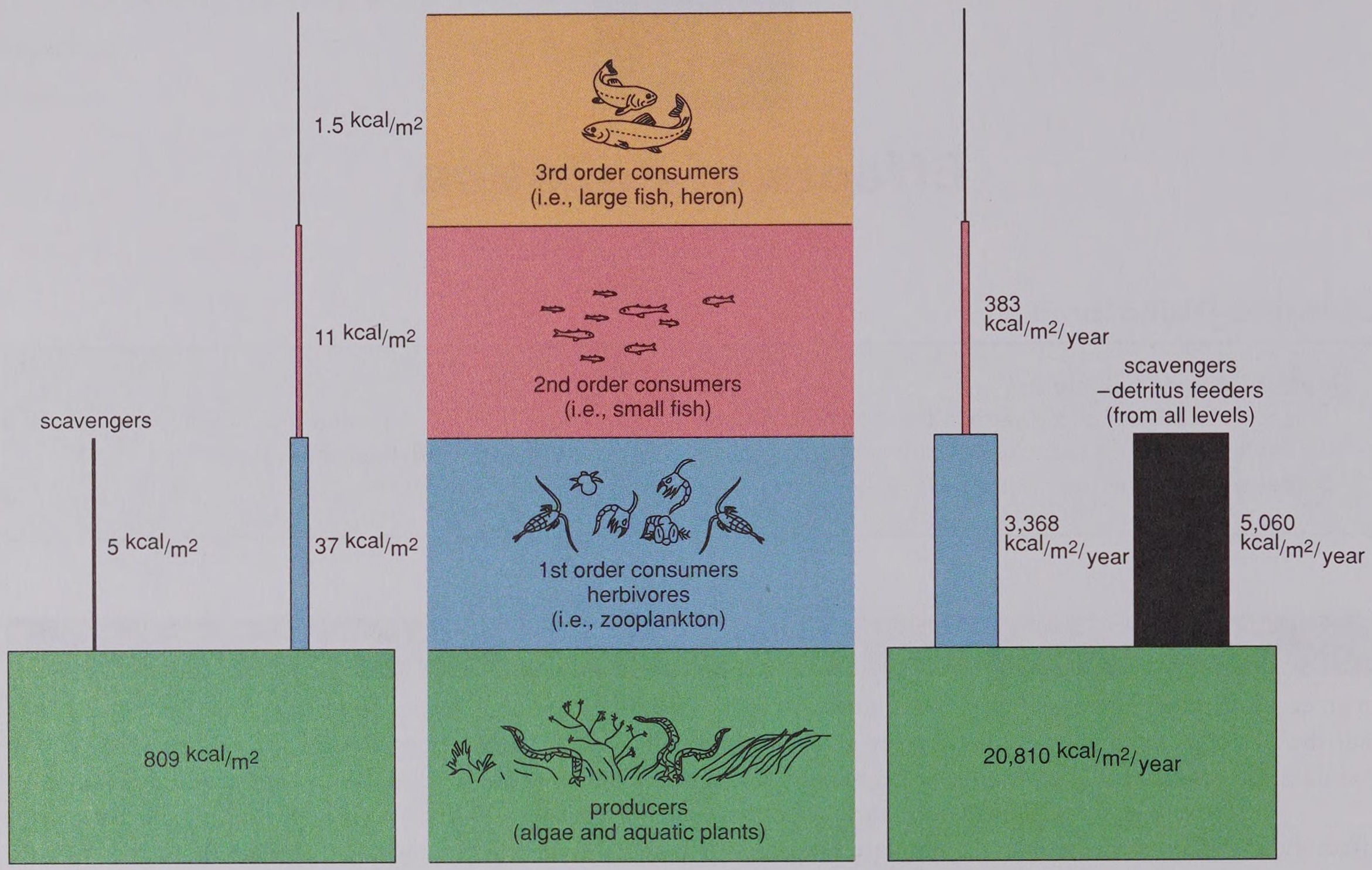
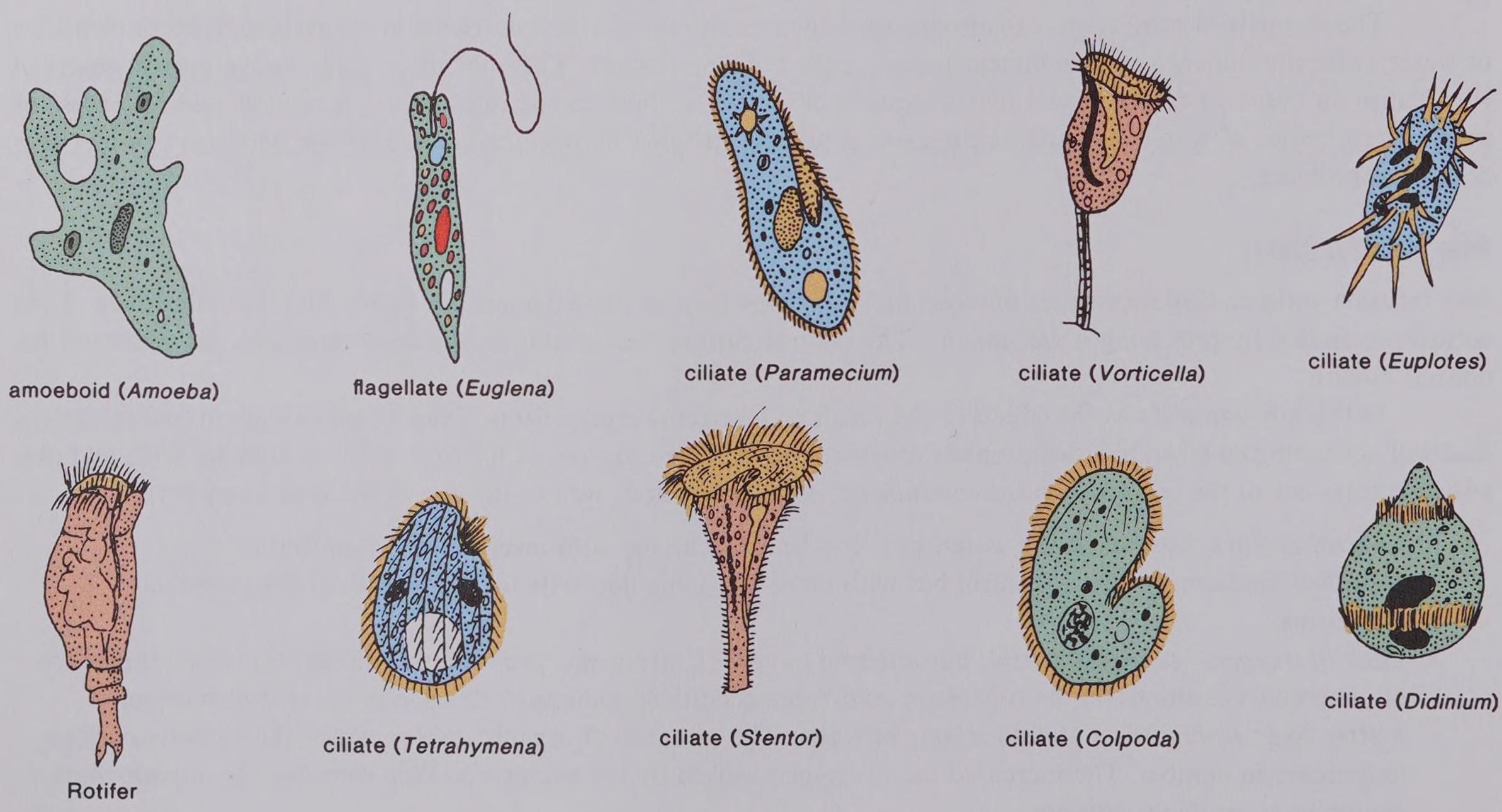


Figure 34.2 Microorganisms found in hay infusion cultures.



4. *Pesticide (low)*—same as control except with the pesticide _____ at a concentration of
(name)

_____
(parts per million—ppm—or percent)

5. *Pesticide (high)*—same as control except with the pesticide _____ at a concentration of
(name)

_____
(parts per million—ppm—or percent)

6. *pH 4*—same as control except it was adjusted to pH 4 with sulfuric acid (H_2SO_4).
7. *pH 2*—same as control except it was adjusted to pH 2 with sulfuric acid (H_2SO_4).

Record in table 34.1 your impression regarding the diversity of life in general and the relative quantity of particular organisms in these cultures.

Table 34.1 Hay Infusion Cultures

Type of Culture	Diversity of Life (list organisms)	Relative Quantity (high, medium, or low)
Control culture		
Enriched medium		
Lack of oxygen		
Pesticide (low)		
Pesticide (high)		
pH 4		
pH 2		

Seed Germination

Just like animals, plant seeds depend on proper conditions of temperature, light, and moisture to germinate, grow, and reproduce. Pollutants affect the ability of seeds to germinate.

1. Observe petri dishes that contain seeds that have been "watered" with solutions that contain *pesticide* (low and high concentrations) and *acid* (low and high concentrations).
2. Record your observations in table 34.2.

Table 34.2 Seed Germination

Conditions	Observation
Pesticide (low)	
Pesticide (high)	
pH 4	
pH 2	

Figure 34.3 *Gammarus.*

Gammarus

Gammarus are *crustaceans,* often called amphipods or scud, that are found in ponds and streams (fig. 34.3).

Control Culture

1. Obtain a prepared control culture in the following manner.
2. Add 50 ml of *control culture water* (spring water) to a container.
3. Measure the pH. _____ Add 4 *Gammarus* to the container.
4. Observe and record the behavior of *Gammarus* for 10–15 minutes.

Where does it spend its time in the container? _____

What percentage of time is spent moving? _____

How does it spend its time? _____

Does it use all of its legs in swimming? _____

Which ones are used in jumping and climbing? _____

Do *Gammarus* avoid each other? _____

What do they do when they "bump" into each other? _____

_____

Thermal Pollution

To simulate thermal pollution, put 100 ml of *water* (after it has been boiled and allowed to cool to 31° C) in a beaker.

Add 2 *Gammarus*. Observe and record any effects on the behavior of the animal. _____

_____

Pesticide Pollution

In this lab, you will test the effects of chemicals on invertebrate organisms. You will use these chemicals in concentrations that will not harm you when handled properly. Please observe all precautions as instructed. Place used pesticide and pH-adjusted solutions in the correct disposal containers. Wash your hands well at the end of the laboratory period.

To test the effect of chemical pollution, use *pesticide-contaminated water.*

1. Place 100 ml of *low-concentration pesticide water* in one beaker and 100 ml of *high-concentration pesticide* in another beaker.
2. Add 2 *Gammarus* to each beaker.
3. Observe and record the effects.

Low concentration of pesticide: _____

_____

High concentration of pesticide: _____

_____

Acid Pollution

To test the effect of acid rain pollution, use *spring water* of approximately pH 4 and pH 2.

1. Test the pH of the control culture and record it here. _____
2. Test and record the pH of the high pH water to be used. _____
3. Test and record the pH of the low pH water to be used. _____
4. Put 100 ml of each of these solutions into beakers and add 2 *Gammarus* to each beaker.
5. Record any effect on the behavior of the organism.

High pH (pH 4): _____

Low pH (pH 2): _____

Synergistic Effects

It has been observed that pollutants sometimes have *synergistic effects;* that is, they combine to give an effect that is greater than the sum of their individual effects. To test the synergistic effects of the variables we have been working with, prepare the following beaker.

1. Add 50 ml of *boiled water* at 31° C to a beaker and 50 ml of the *pesticide-contaminated water* to the same beaker. (Use the high-concentration pesticide water.)
2. Adjust the pH to 2 using 5% *sulfuric acid* (H_2SO_4). *Caution:* Be very careful using the acid. If the acid spills, immediately remove it from your skin, clothing, etc. Add 2 *Gammarus,* observe the effects and record them here:

_____

_____

Summary

Look again at figure 34.1 and hypothesize how pollutants might affect the food chain represented. _____

_____

One additional effect should be kept in mind. Poisonous organic chemicals, represented in today's laboratory by a pesticide, sometimes are subject to *biological magnification;* that is, they concentrate as they are passed from one trophic level to the next because they are not excreted to any great extent. Since humans are at the ends of food chains, they, like top predators, are most subject to the effects of biological magnification.

Cultural Eutrophication

Inorganic nutrients that enter a body of water support the growth of free-floating algae called *phytoplankton.* Normally, the populations of algae are kept under control because they are fed upon by free-swimming animals called *zooplankton.* If they are not kept under control, they increase to the extent that, when they die off, the decomposing bacteria use up all the available oxygen. Now the fish die, and we say the water is polluted. One representative zooplankton that keeps algae under control is *Daphnia.*

Experimental Procedure

Ⓢ 1. Fill one spectrophotometer tube (*cuvette*) with a well-stirred culture of algae.
Ⓢ 2. Add 10 starved *Daphnia,* and immediately measure the absorbance with the spectrophotometer, following the instructions given by your instructor.
Ⓢ 3. Record the absorbance reading in the "Absorbance before feeding" space following.
Ⓢ 4. Note that absorbance serves as a measure of the algal population level since the greater the number of algal cells, the greater the absorbance. The higher the absorbance the greater the amount of light absorbed and *not* passed through the solution. If a *Daphnia* swims through the beam of light, a strong deflection should occur; do not use any such higher figure but use the lower figure for the absorbance.
Ⓢ 5. Remove the cuvette with the *Daphnia* to a safe place in a test tube rack. Allow the *Daphnia* to feed for 30 minutes.
6. Measure the absorbance again and record in the "Absorbance after feeding" space.

Absorbance before feeding _____

Absorbance after feeding _____

Observation of *Daphnia* (Optional)

Ⓢ 1. Construct a small pool out of petroleum jelly in the center of a small petri dish.
Ⓢ 2. Take a *Daphnia* from the stock culture, place it on its back in the petroleum jelly, and observe it under the binocular dissecting microscope.
Ⓢ 3. Note the clamlike carapace and the legs waving rapidly as they filter the water.
Ⓢ 4. Add a drop of *carmine* solution and observe how the "food" is filtered from the water and passed through the gut. The gut is more visible if you push the animal onto its side. In this position, you may also observe the heart beating in the region above the gut and just behind the head.
Ⓢ 5. Allow the *Daphnia* to filter-feed for up to 30 minutes, and observe the progress of the carmine particles through the gut.

Does the carmine travel completely through the gut in 30 minutes? _____
The spectrophotometer cannot tell the difference between live algal cells and fecal particles.
If the food retention time in the gut is less than 30 minutes, how would this bias the results of your measure of absorbance previously? _____

_____

Problem

Some developers want to build condominium units on the shores of Silver Lake. Homeowners in the area have asked the regional council to determine how many units can be built without altering the nature of the lake. You are a member of the council who has been given appropriate information on which to make your decision. You have been informed of the following:

1. The present population of *Daphnia* is 10 animals/liter, and the population presently filters 24% of the lake per day, meaning that it removes this percentage of the algae population per day. This is sufficient to keep the lake essentially clear. Predation will allow the *Daphnia* population to increase no more than another 50 animals/liter. Therefore, 50 *Daphnia*/liter will be available for feeding on the increase in algae that would result from the building of the condominiums.
   Ⓢ Using this information, fill in table 34.3.

Table 34.3 *Daphnia* Filtering	
No. of *Daphnia*/liter	**% Lake Filtered**
10	24%
50	

2. The sewage system of the condominiums will add nutrients to the lake. Phosphorous output would be 1 kg per day for every 10 condominiums, and this will cause a 30% per day increase in algal population.
   Using this information, complete table 34.4.

Table 34.4 Cultural Eutrophication		
No. of Condos	**Phosphorus Added**	**Increase in Algae Population**
10	1 kg	30%
20		
30		
40		
50		

Assume that phosphorus is the only nutrient that will cause an increase in algal population and that *Daphnia* is the only type of zooplankton available to feed on the algae.

How many condominiums would you allow the developer to build? _____

Laboratory Review 34

_____ 1. What type of pollution results when water from rivers and ponds is used for cooling industrial processes?

_____ 2. Which has the higher hydrogen ion concentration, pH 2 or pH 4?

_____ 3. Which organisms studied today are producers in food chains?

_____ 4. Which organisms studied today are consumers in food chains?

_____ 5. In a balanced ecosystem, which trophic level is expected to have the largest populations?

_____ 6. Overenrichment of a body of water due to human activities is called what?

7. What happens when algae overincrease?

8. What is biological magnification?

9. Many industrial processes produce large amounts of oxides of nitrogen and sulphur. When these waste products are dumped into the atmosphere, they combine with water vapor to produce nitrous acid and sulfuric acid. Wherever these acids are washed out of the atmosphere, the rain will become acidic. Does this affect aquatic organisms?

References

Stubbs, H. S. 1983. Acid precipitation awareness curriculum materials in the life sciences. *American Biology Teacher* 45(4): 217.

Snow, B.L., ed. *Biological Laboratory Exercises*. Boston, MA: Suffolk University.

Appendix

A

Preparing a Laboratory Report/Laboratory Report Form

A laboratory report has the sections (and paragraphs) noted following. You may use this description and a copy of the worksheet to help you write a report assigned by your instructor.

1. Introduction. The introduction tells the reader what the experiment was about.

   a. Background information. Begin by giving an overview of the topic.

      Look at the introduction to the laboratory (and/or at the introduction to the section for which you are writing the report). *Do not copy the information* but use it to give you ideas about what information to include.

      For example, suppose you were doing a laboratory report on the "Role of Light," Laboratory 4. You might want to give a definition of photosynthesis and explain the composition of white light.

   b. Purpose. Next, state what the experiment was about.

      Think about the steps of the experiment and then decide what its purpose was. Continuing with the previous example, the experiment tested the effect of white and green light on photosynthesis—this tells you what the purpose was.

      Therefore, you might state that the purpose of this experiment was to determine the effect of white light versus green light on the photosynthetic rate.

   c. Hypothesis. Finally, hypothesize what you expected the results to be.

      Think about what results you expected from the experiment. Most likely the introduction to the laboratory (and/or the particular section) will give you a hint as to the expected results. State this in the form of a hypothesis.

      For example, you might state it was hypothesized that white light would be more effective for photosynthesis than green light.

2. Procedure. The procedure tells the reader how you did the experiment.

   a. Equipment used. This paragraph should help the reader envision the setup of the experiment.

      Look at any illustrations that accompany the experiment. For example, look at figure 4.3 in the laboratory manual and read the paragraph that describes how the experiment is to be conducted. Now describe the equipment in your own words.

      For example, you might state that a 150-watt lamp was the source of white light directed at *Elodea* placed in a test tube filled with a solution of $NaHCO_3$. A beaker of water placed in between the lamp and test tube was a heat absorber.

   b. Collection of data. Tell the reader how the experiment was done.

      Think about what you did during the experiment. What did you observe or what did you measure?

      For example, you might state that the rate of photosynthesis was determined by the amount of oxygen released and was measured by how far water moved in a side arm of the test tube.

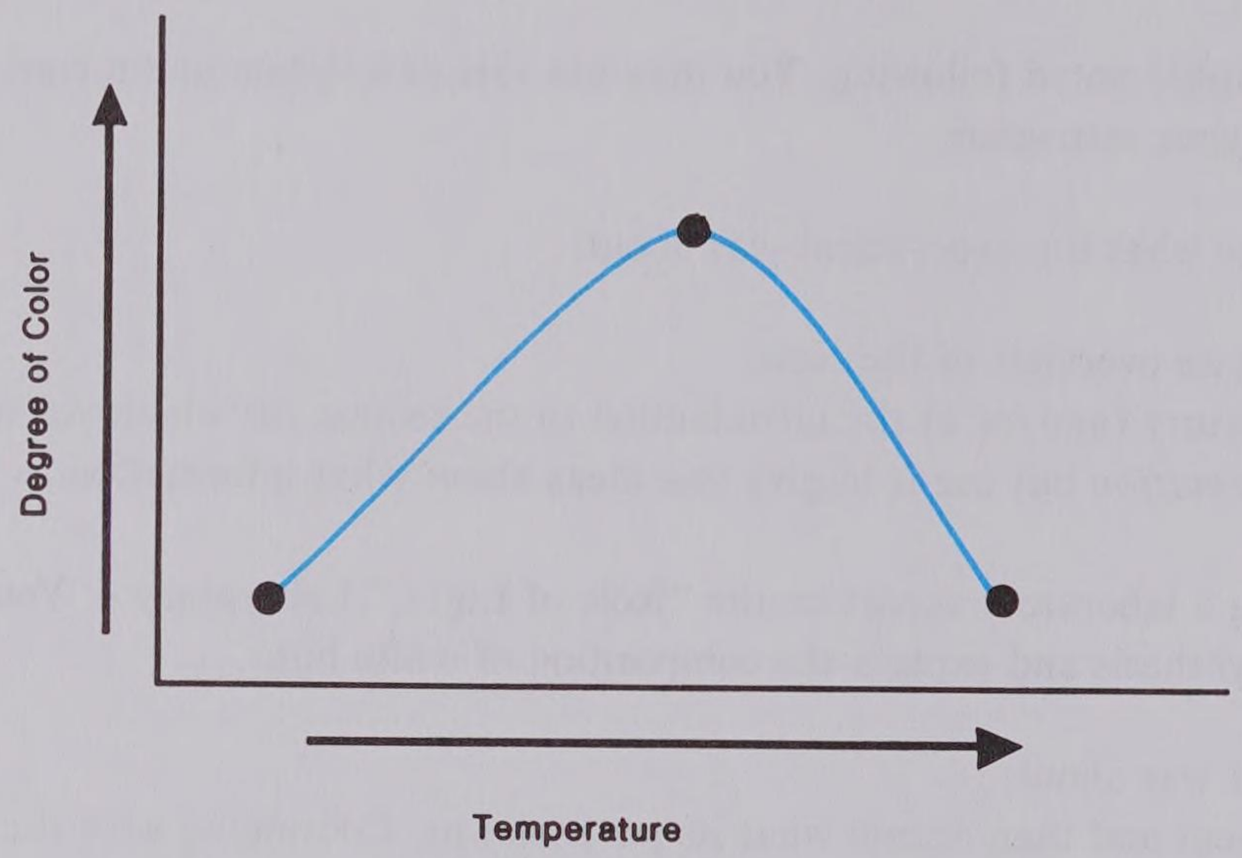
3. Results. The results present the data in a clear manner.

   a. Graph or table. If at all possible, show your data as a graph or in a table.

      Look at any tables you may have filled in while you were in the laboratory and reproduce those sections that pertain to the collection of data. (Do not include any columns that have to do with interpretation.)

      For example, if you were writing a laboratory report on the "Role of Light" you could reproduce table 4.1 for this section.

      It is also possible that you might be able to draw a graph depicting the results. For example, you could draw a graph for the results you recorded in table 5.2. It might look like this:



   b. Description of data.

      Look at the data in your table and/or graph and decide what it tells you.

      For example, for table 4.1, you might state that the data in the table indicates that there was a faster rate of photosynthesis with white light than with green light.

4. Discussion. In this section the data is interpreted and any conclusions are given.

   a. Support of hypothesis. Tell whether the hypothesis was supported.

      Consider your hypothesis once more and compare it to your data. Does your data agree with the hypothesis?

      For example, you might state that the results supported the hypothesis that white light gives a higher rate of photosynthesis than green light.

   b. Explanation. Explain as far as possible why you think you got the results you did.

      Look at any questions you answered while in the laboratory and use them to help you decide on an appropriate explanation.

      For example, see the "Role of Light" section in Laboratory 4. The answers to the questions might help you state that white light gives a higher rate of photosynthesis because it contains all the visual light rays. Green light gives a lower rate of photosynthesis because green plants do not absorb green light.

      (You should be sure to report your data exactly. If your results fail to support the hypothesis, explain why this may have occurred now.)

   c. Conclusion. In general terms tell what you learned from the experiment.

      Review the entire experiment in your mind and decide what you learned from the experiment.

      For example, you might state that it was concluded that the quality of light can affect the rate of photosynthesis.

Points to Remember

1. *Clarity for the Reader.* Your report should be organized and presented so the reader can easily understand your work.
2. *Good Writing.* Give particular attention to grammar and style. Your writing will be enhanced by careful proofreading and revision. Have someone else read and critique a draft of your report. Then consider their comments seriously in your revisions.
3. *Observations and Interpretations.* The distinction between observations and interpretations should be kept clear. These are frequently confused in student reports.
4. *Exercise Good Judgment.* As you are preparing your report, you will constantly be making difficult decisions about how best to present and interpret your data. Your attention to these decisions will be reflected in the quality of your report.

Date _____

Name _____

Laboratory report for _____

_____

1. Introduction

    a. Background information

    b. Purpose

    c. Hypothesis

2. Procedure

    a. Equipment used

    b. Collection of data

3. Results

a. Graph or table
(Place these on attached sheets.)

b. Description of data

4. Discussion

a. Support of hypothesis

b. Explanation

c. Conclusion

Appendix

B

Metric System

The International System of Units (SI) has recognized 7 base units of measurement, 5 of which are of interest to general biology students: meter (length), kilogram (mass), second (time), degree Celsius (temperature), and mole (amount of substance).

Length

Unit	*Value*	*Symbol*
kilometer	1,000 meters	km
meter	base unit	m
decimeter	0.1 meter	dm
centimeter	0.01 meter	cm
millimeter	0.001 meter	mm

Volume

Volume is the 3-dimensional space occupied by a material; therefore, volume is measured by these units:

Unit	*Value*	*Symbol*
cubic meter	derived unit	m^3
cubic decimeter	0.001 cubic meter	dm^3
cubic centimeter	0.000 001 cubic meter	cm^3
cubic millimeter	0.000 000 001 cubic meter	mm^3

Very often, however, the unit called a liter is used for liquid measurement in science. A liter (l) is equal to a cubic decimeter, and a milliliter (ml) is equal to a cubic millimeter.

Mass

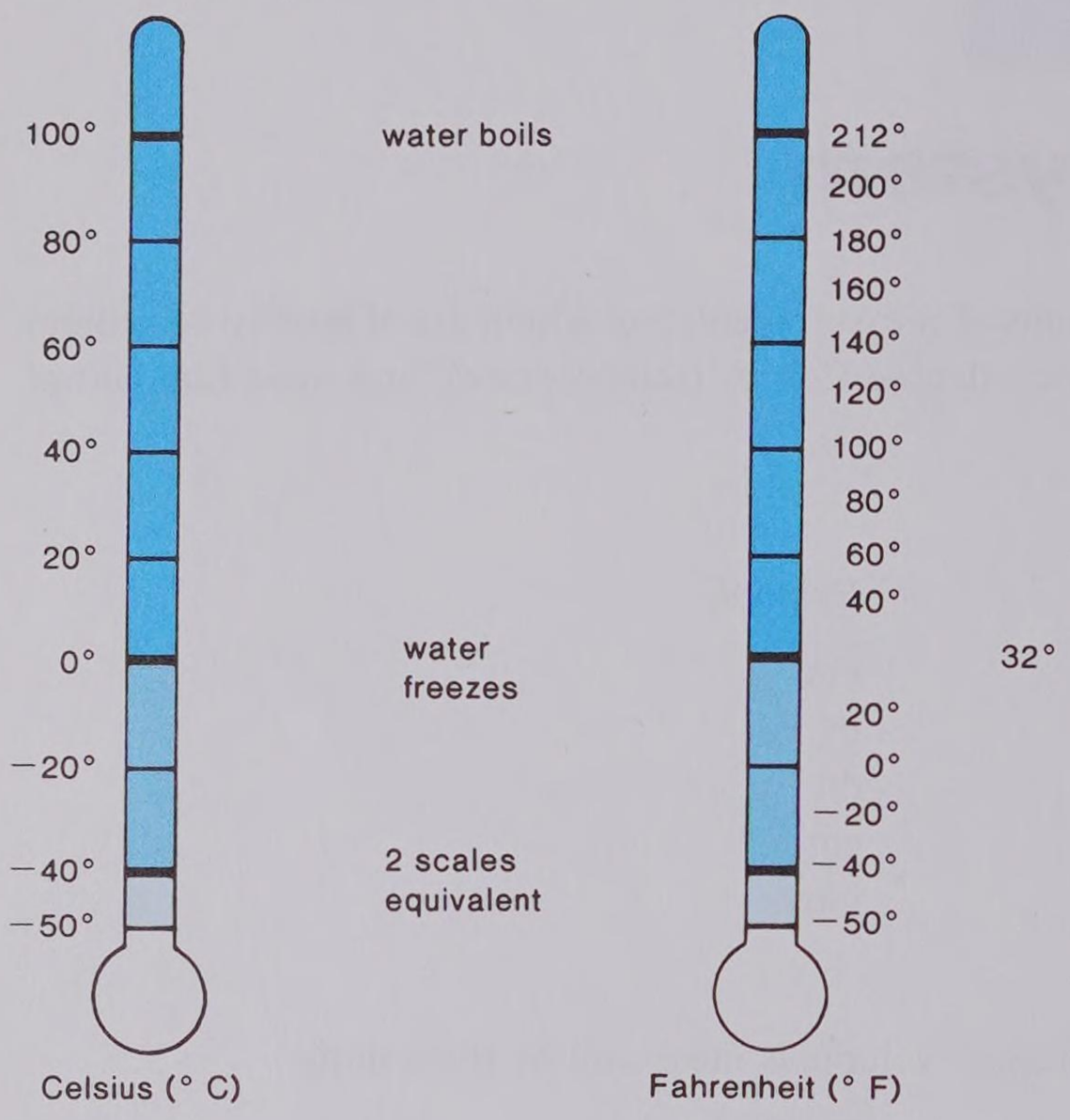
Unit	*Value*	*Symbol*
kilogram	base unit	kg
gram	0.001 kg	g
decigram	0.1 g	dg
centigram	0.01 g	cg
milligram	0.001 g	mg

Time

Unit	*Value*	*Symbol*
second	base unit	s
minute	60 s	min
hour	3, 600 s	h
day	86, 400 s	d

Temperature

To change degrees Fahrenheit to degrees Celsius, subtract 32 from the Fahrenheit temperature and multiply by 5/9. To convert from Celsius to Fahrenheit, multiply the Celsius temperature by 9/5 and add 32.



The term Centigrade is very often used instead of Celsius.

Mole

Mass refers to the total quantity of substance and amount of substance; *mole* refers to the concentration of substance. The official SI definition of mole (mol) is "the amount of substance of a system which contains as many elementary entities as there are atoms in 0.012 kilogram of carbon-12." Mole is the unit of measurement to be used when comparing the concentration of atoms or chemicals.

Appendix

C

Classification of Organisms

The classification system given here is a simplified one, containing all the major kingdoms, as well as divisions (called phyla in the kingdom Protista and kingdom Animalia).

Kingdom Monera

Prokaryotic, unicellular organisms. Nutrition principally by absorption, but some are photosynthetic or chemosynthetic.

- Division Archaebacteria: methanogens, halophiles, and thermoacidophiles
- Division Eubacteria: all other bacteria, including cyanobacteria (formerly called blue-green algae)

Kingdom Protista

Eukaryotic, unicellular organisms (and the most closely related multicellular forms). Nutrition by photosynthesis, absorption, or ingestion.

- Phylum Sarcodina: amoeboid protozoans
- Phylum Ciliophora: ciliated protozoans
- Phylum Zoomastigina: flagellated protozoans
- Phylum Sporozoa: parasitic protozoans
- Phylum Chlorophyta: green algae
- Phylum Pyrrophyta: dinoflagellates
- Phylum Euglenophyta: *Euglena* and relatives
- Phylum Chrysophyta: diatoms
- Phylum Rhodophyta: red algae
- Phylum Phaeophyta: brown algae
- Phylum Myxomycota: slime molds
- Phylum Oomycota: water molds

Kingdom Fungi

Eukaryotic organisms, usually having haploid or multinucleated hyphal filaments. Spore formation during both asexual and sexual reproduction. Nutrition principally by absorption.

- Division Zygomycota: black bread molds
- Division Ascomycota: sac fungi
- Division Basidiomycota: club fungi
- Division Deuteromycota: imperfect fungi, i.e., means of sexual reproduction not known

Kingdom Plantae

Eukaryotic, terrestrial, multicellular organisms with rigid cellulose cell walls and chlorophylls *a* and *b*. Nutrition principally by photosynthesis. Starch is the food reserve.

- Division Bryophyta: mosses and liverworts
- Division Psilophyta: whisk ferns
- Division Lycophyta: club mosses
- Division Sphenophyta: horsetails
- Division Pterophyta: ferns
- Division Cycadophyta: cycads
- Division Ginkgophyta: ginkgos
- Division Gnetophyta: gnetae
- Division Coniferophyta: conifers

Division Anthophyta: flowering plants
Class Dicotyledonae: dicots
Class Monocotyledonae: monocots
Eukaryotic, usually motile, multicellular organisms without cell walls or chlorophyll.
Nutrition principally ingestive, with digestion in an internal cavity.
Phylum Porifera: sponges
Phylum Cnidaria: radially symmetrical marine animals
Class Hydrozoa: hydras. Portuguese man-of-war
Class Scyphozoa: jellyfishes
Class Anthozoa: sea anemones and corals
Phylum Platyhelminthes: flatworms
Class Turbellaria: free-living flatworms
Class Trematoda: parasitic flukes
Class Cestoda: parasitic tapeworms
Phylum Nematoda: roundworms
Phylum Rotifera: rotifers
Phylum Mollusca: soft-bodied, unsegmented animals
Class Polyplacophora: chitons
Class Monoplacophora: *Neopilina*
Class Gastropoda: snails and slugs
Class Bivalvia: clams and mussels
Class Cephalopoda: squids and octopuses
Phylum Annelida: segmented worms
Class Polychaeta: sandworms
Class Oligochaeta: earthworms
Class Hirudinea: leeches
Phylum Arthropoda: animals with chitin exoskeleton and jointed appendages
Class Crustacea: lobsters, crabs, barnacles
Class Arachnida: spiders, scorpions, ticks
Class Chilopoda: centipedes
Class Diplopoda: millipedes
Class Insecta: grasshoppers, termites, beetles
Phylum Onychophora: small, sluglike animals with legs; internal features of both annelids and arthropods
Phylum Echinodermata: marine; spiny, radially symmetrical animals
Class Crinoidea: sea lilies and feather stars
Class Asteroidea: sea stars
Class Ophiuroidea: brittle stars
Class Echinoidea: sea urchins and sand dollars
Class Holothuroidea: sea cucumbers
Phylum Chordata: dorsal supporting rod (notochord) at some stage; dorsal hollow nerve cord; pharyngeal pouches or slits
Subphylum Urochordata: tunicates
Subphylum Cephalochordata: lancelets
Subphylum Vertebrata: vertebrates
Class Agnatha: jawless fishes (lampreys, hagfishes)
Class Chondrichthyes: cartilaginous fishes (sharks, rays)
Class Osteichthyes: bony fishes
Subclass Sarcopterygii: lobe-finned fishes
Subclass Actinopterygii: ray-finned fishes
Class Amphibia: frogs, toads, salamanders
Class Reptilia: snakes, lizards, turtles

Class Aves: birds
Class Mammalia: mammals
Subclass Prototheria: egg-laying mammals
Order Monotremata: duckbilled platypus, spiny anteater
Subclass Metatheria: marsupial mammals
Order Marsupialia: opossums, kangaroos
Subclass Eutheria: placental mammals
Order Insectivora: shrews, moles
Order Chiroptera: bats
Order Edentata: anteaters, armadillos
Order Rodentia: rats, mice, squirrels
Order Lagomorpha: rabbits, hares
Order Cetacea: whales, dolphins, porpoises
Order Carnivora: dogs, bears, weasels, cats, skunks
Order Proboscidea: elephants
Order Sirenia: manatees
Order Perissodactyla: horses, hippopotamuses, zebras
Order Artiodactyla: pigs, deer, cattle
Order Primates: lemurs, monkeys, apes, humans
Suborder Prosimii: lemurs, tree shrews, tarsiers, lorises, pottos
Suborder Anthropoidea: monkeys, apes, humans
Superfamily Ceboidea: New World monkeys
Superfamily Cercopithecoidea: Old World monkeys
Superfamily Hominoidea: apes and humans
Family Hylobatidae: gibbons
Family Pongidae: chimpanzees, gorillas, orangutans
Family Hominidae: *Australopithecus**, *Homo erectus**, *Homo sapiens sapiens*

*extinct

Appendix

D

List of Materials

Laboratory 1 Microscopy and Linear Metric Measurement

Hardware

Alcohol swabs (ethyl alcohol), ethanol, 70% (Carolina 86–1261, 86–1263, 86–1265) and cotton applicators (Carolina 64–6580)
Droppers—dropping pipettes, all plastic (Carolina 73–6892 to 73–6899) or dropping pipettes, medicine droppers (Carolina 73–6900 to 73–6912)
Microscopes, compound light
Microscope coverslips, plastic (Carolina 63–2900) or glass coverglasses (Carolina 63–3008 to 63–3478)
Microscope, binocular dissecting and illuminator
Microscope slides, glass (Carolina 63–1920, 63–1930, 63–2000, 63–2010, 63–2020)
Paper, lens, lens paper booklets (Carolina 63–4000, 63–4005) or as unbounded sheets (Carolina 63–4010, 63–4016, 63–4030, 63–4032, 63–4034) and cut into 5–10 cm squares
Rulers, millimeter, clear plastic—transparent vinyl ruler (Carolina 70–2604)
Scalpels (Carolina 62–5920 to 62–6524) and scalpel blades (Carolina 62–6590 to 62–6599)
Toothpicks, flat

Microscope Slides, Prepared

Colored threads (Carolina BM 10)
or
Three colors of sewing thread
Letter e (Carolina BM 1)

Chemicals

Iodine solution (IKI)
Protoslo (Carolina 88–5141)
or
Methyl cellulose, solution (Carolina 87–5181 or 87–5161)

Living Material

Onions
Pond water
or
Paramecium, live culture (Carolina L2, L2A, or L2C)

Texts

Pictorial guides to aquatic organisms: *A Key to Freshwater Biology* by Needham and Needham

Laboratory 2 Chemical Composition of Cells

Hardware

Droppers (dropping bottles)
Knives and cutting board
Marking pencils (wax or lab markers)
Microscopes, compound light
Microscope coverslips
Mortar and pestle
Razor blades
Paper, brown (squares 4 cm × 4 cm)
Test tubes
Water bath, boiling

Microscope Slides, Prepared

Adipose tissue slide (Carolina H 6250)

Chemicals

Alpha amylase solution (1%) (Carolina 20–2350)
Albumin solution (1%) (Carolina 84–2250)
pH 7 buffer (Carolina 84–9380)
Benedict's reagent powder (Carolina 84–7091)
Bile salts (1%) (Carolina 79–5000) or Tween (1%) (Carolina 20–7861)
Biuret reagent (Carolina 84–8213)
Glucose solution (1%) (Dextrose) (Carolina 85–7430)
Iodine solution (IKI)
Potassium iodide (Carolina 88–3790)
Iodine (Carolina 86–8970)
Hydrochloric acid (HCl) (0.2%) (Carolina 86–7793)
Pepsin (1%–2%) (Carolina 87–9378)
Starch solution (1%) (Carolina 89–2530)
Water, distilled

Fresh Materials

Bread, moist
Flour and water mixture
Margarine
Milk (skim)
Onion
Orange juice
Potato
Syrup from canned fruit
Vegetable oil
Everyday materials

Laboratory 3 Cell Structure and Function

Hardware

Dropping bottles
Droppers
Forceps—dissecting forceps, fine points, chrome (Carolina 62–4022)
Osmosis apparatus
Osmometer
Microscope, compound light
Microscope coverslips, plastic (Carolina 63–2900)
Microscope slides, glass (Carolina 63–2000)
Ruler, 12 in plastic with metric measurement for measuring osmometer
Test tubes—culture tubes, bacteriological, screw cap, Pyrex 16 mm × 150 mm (Carolina 73–1520a) for diffusion with gelatin or agar
Test tubes, screw cap (Carolina 73–1520a) or test tubes, Pyrex 16 mm × 150 mm (Carolina 73–1014) and laboratory rubber stoppers, solid, size 1 (Carolina 71–2402) for tonicity of blood

Microscope Slides, Prepared

Squamous epithelium—frog skin, outer layer, flat mount (Triarch HA 1–11); epithelium squamous, flat mount of amphibian epidermis (Turtox H1.121)

*See *Laboratory Resource Guide* for further instructions.

Pseudostratified columnar ciliated epithelium (Carolina 99–6012)
or
Trachea, human, cross section and longitudinal section (Carolina H 7700)
Fresh epidermis of _____ or Privet leaf section, parallel to epidermis (Carolina 97–8702)

Models

Supplemental if available:
Animal cell (Carolina 56–3950, 56–3960, 56–3961, 56–3962)
Leaf (Carolina 56–8801, 56–8812, 56–8810)
Plant cell (Carolina 56–8050)
Pseudostratified columnar epithelium
Squamous epithelium

Chemicals

Food color, blue (or other dark color); purchase locally in gelatin tubes
Gelatin, powder (Carolina 86–4658, 86–4660) or agar, powder (Carolina 84–2131); 2% solution
Corn syrup (Karo), 50%; osmosis demonstration
Potassium permanganate ($KMnO_4$) (Carolina 88–4130); solid crystals
Sodium chloride (NaCl) (Carolina 88–8901); 0.9% and 10%
Spray, scented aerosol; purchase locally
Water, distilled

Living Material

Amoeba, live cultures (Carolina L1)
Elodea, living (Carolina 16–2100)
Sheep blood, pooled, citrated (Carolina 82–8950, 82–8954, 82–8960); also available from Harvey Media

Laboratory 4 Photosynthesis

Hardware

Aquarium aerator—plastic air stone (Carolina 67–1792)
Aluminum foil to wrap test tubes
Beakers, 1,000 ml (Carolina 71–7910)
Chromatography apparatus
Chromatography paper, Whatman #1; 12 cm × 12 cm square sheets (Carolina 68–9110)
Hood, fume
Lamps, desk—or gooseneck
Mortar and pestle (Carolina 74–2880 to 74–2886)
Prism (Carolina 75–4932) or spectroscope (Carolina 75–5318 to 75–5335)
Pipettes, capillary—disposable glass Pasteur pipette (Carolina 73–6060) or glass capillary tubes (71–1040)
Razor blades, sharp single edge (Carolina 62–6930)
Ruler, clear vinyl (Carolina 70–2604)
Scissors, dissecting, chrome (Carolina 67–1759)
Straws
Test tube racks, large tube, 25 mm holes (Carolina 73–1876) or ring stand and clamp
Volumeter apparatus
Wax pencil

Chemicals

Acetone (Carolina 84–1481, 84–1483)
Chromatography solution; 45 parts petroleum ether (Carolina 87–9540, 87–9542) and 5 parts acetone
Food coloring, green (optional)
Phenol red solution, 0.04%, powder (Carolina 87–9850)
Sodium bicarbonate ($NaHCO_3$), 3% (aerated), powder (Carolina 88–8360)

Fresh Material

Elodea
Spinach, washed and drained or geranium or *Coleus* leaves

Laboratory 5 Enzymes

Hardware

Boiling water bath 100° C
Dropping bottles (Carolina 71–6520)
Incubator at 37° C
or
Warm water bath
Pencils, wax marking (Carolina 65–7730)
Refrigerator at 5° C or ice water bath
Test tube clamps
Test tubes, standard
Test tube racks

Chemicals

Phenylthiourea, 0.01% (Aldrich 13,501–1); "Inhibitor"
Pyrocatechol, 0.66% (Aldrich 13,501–1); "Substrate"
Sucrose, 5%; "Alternate substrate"
Tyrosinase, 0.05% (Sigma T 7755) (or potato juice); "Enzyme"
Water, distilled, adjusted to pH 3, 7, 11

Optional

Glucose solution, 2%
Respirometers
Sodium fluoride (NaF) 0.02 M
Yeast suspension (of baker's yeast)
Warm water bath (incubator substitute)

Laboratory 6 Mitosis and Meiosis

Hardware

Models, mitosis and meiosis (Carolina 56–1608 to 56–1650); oogenesis is included in Human Cleavage Set (Carolina 56–3102)
Microscopes, compound light
Paper, lens (Carolina 63–4000 to 63–4034)

Microscope Slides, Prepared

Allium (onion) mitosis, root tip (Carolina 97–8093T, B552, B552F, and B553)
Ascaris oogenesis, *Ascaris* ova (Carolina E380)
Whitefish blastula, whitefish mitosis (Carolina E1025)
or
Slide Set 28 Whitefish and Onion Mitosis
or
Slide Set *Ascaris,* Whitefish and Onion Mitosis

Preserved Material

Ascaris demonstration of dissected female (Carolina P265C)

Optional—Duration of Mitotic Stages

Allium mitosis slide (see previous)
Calculators

Optional—Broad Bean, Squash Preparation

Broad bean seed (Carolina 15–8302) (see following for germination directions)
Fixing and staining ingredients
Aceto-carmine, Schnieders (Carolina 84–1421, 84–1423, 84–1425)
Ethanol, 70%, not denatured (Carolina 86–1261, 86–1263, 86–1265)
Ethanol: glacial acetic acid fixative, 3:1 Acetic acid, glacial (Carolina 86–1292)
Ferric ammonium citrate (Carolina 86–2330)
Hydrochloric acid, 1N (Carolina 86–7843)
Para-dichlorobenzene, saturated; p-dichlorobenzene (Carolina 85–7809)
Forceps, fine point (Carolina 62–4022)
Small vials or tight-capped containers
Bottles, small 200–300 ml with tight-fitting screw caps

*See *Laboratory Resource Guide* for further instructions.

Laboratory 7 Mendelian Genetics

Hardware

Brushes, camel hair—*Drosophila* sorting brush (Carolina 17–3095)
Culture vial (Carolina 17–3085)
Culture vial plug (Carolina 17–3086)
File cards, white, 3 × 5
FlyNap Kit (Carolina 17–3010); one bottle plus 12 anesthetic wands
or
FlyNap (Carolina 17–3025) and anesthetic wands (Carolina 17–3027)
or
Drosophila anesthetizer (Carolina 17–3040) for use with FlyNap or ether for anesthesia (Carolina 86–1370); *extremely flammable!*
Labels (Carolina 65–7340)
Microscopes, binocular dissecting
Microscope slides
Petri dishes, disposable polystyrene (Carolina 74–1346)
Tobacco seedling

Chemicals

Agar, powder (Carolina 48–2131); 1.5% in petri dishes for tobacco seedling
Drosophila instant medium—formula 4–24 plain (Carolina 17–3200)
Sodium chloride (NaCl), 0.7% (Carolina 88–8901)
Yeast, dry; one packet of dry baker's yeast

Living Material

Drosophila melanogaster cultures (with all stages)
Wild-type (Carolina 17–2100)
Vestigial (Carolina 17–2460)
Ebony (Carolina 17–2500)
White (Carolina 17–2220)
Genetic corn ears—Corn dihybrid genetics biokit (17–6380)
or
Corn ears only, R & Su Alleles (purple:yellow; starchy: sweet) (Carolina 17–6600)
Tobacco seeds, green:albino, 3:1 (Carolina 17–8400)
or
Albino tobacco BioKit (17–8300) contains all materials necessary to germinate seeds

Optional—Drosophila *Chromosomes*

Aceto-orcein stain, 2% (Carolina 84–1541)
Drosophila mojavensis larvae (Carolina 17–2870)
Drosophila salivary chromosomes prepared slide (Carolina G340) (optional)
Microscopes, compound light
Microscope coverslips
Microscope slides
Needles, dissecting, stainless steel (Carolina 62–7220)
or
One needle and one forceps, extra fine point (Carolina 62–4684)
Sealant, temporary—vaseline, wax, or clear nail polish
Toothpicks, flat—sealant applicator; one box/classroom
Towels, paper

Laboratory 8 Human Genetics

Hardware

Ichikawa color blindness test booklets (Carolina 69–4620)

Laboratory 9 Molecular Genetics

Hardware

Aluminum foil or black construction paper; darken developing jars
Centrifuge
Centrifuge tubes
Chromatography paper, Whatman #1
Developing solution, eye pigment: 25 ml/developing jar
Developing jars, darkened with aluminum foil
Dissecting needles
DNA model or DNA-RNA model kits (Carolina—see Genetics section)
Droppers
Glass rods
Glasses, safety
Graduated cylinders, 10 ml
Hood
Lamp, ultraviolet, mineral light or view box
Microscopes, compound light
Microscope coverslips
Microscope slides
Mortar and pestle
Pencils, regular
Pencils, set of colored (optional)
Pencils, wax or lab marking
Petri dishes, disposable polystyrene, sterile (Carolina 74–1350)
Razor blades
Rulers
Stapler
Test tubes
Test tube racks
Towels, paper
Water bottles

Microscope Slides, Prepared

Blood, human, normal (Carolina H–6450)
Blood, human, sickle-cell disease (Carolina PH–1015)

Chemicals

Agar (Carolina 79–6201)
Glucose-1-phosphate (Carolina 68–5372)
Iodine solution
Water, distilled

Living Material

Drosophila melanogaster cultures:
Wild-type; red-eyed (Carolina 17–2100)
White-eyed (Carolina 17–2220)
Sepia-eyed (Carolina 17–2575)

Fresh Material

Peas (both dry and soaked):
Smooth (Alaska); green, smooth, tall (Carolina 15–8863)
Wrinkled (Little Marvel): green, wrinkled, dwarf (Carolina 15–8883)

Laboratory 10 Biotechnology—Gene Cloning

Hardware

Beaker, 50 ml
Bunsen burner
Centrifuge, micro
Ice bath
Incubator (37° C)
Microcentrifuge
Microcentrifuge tubes, 1.5 ml
Micropipetters (5μl and 100μl)
Micropipetter, tips
Pencils, wax or marking
Spreader
Water baths (65° C, 42° C, 37° C)

Chemicals

Alcohol, for spreader sterilization
Nutrient agar plates, labeled AMP
Nutrient agar plates plus ampicillin, labeled AMP
Reaction buffer 10×
Restriction enzyme *Eco*RI or *Bam*HI

Living Material

E. coli cells, competent

*See *Laboratory Resource Guide* for further instructions.

Laboratory 11 Biotechnology—Gel Electrophoresis

Hardware

Electrophoresis Apparatus:
  Power supply
  Casting tray
  Electrophoresis chamber
  Cables
Micropipettes
Micropipette tips

Chemicals

Agarose powder
TE buffer (Tris/EDTA) electrophoresis buffer
Protein sample kit
Separation of Plasmid DNA Samples (Optional)
Plasmid DNA (pBR322 or pBR325) from Laboratory 10
Tube A (restricted plasmid DNA) from Laboratory 10
Tube B (ligated plasmid DNA) from Laboratory 10
TE buffer (Tris/EDTA) from Laboratory 10
Ethidium bromide (*Caution:* toxic) or methylene blue
Ultraviolet viewing tray or light

Laboratory 12 Evidence of Evolution

Hardware

Fossils, box, or representative fossils from the geological eras (Cenozoic, Mesozoic, Paleozoic)
Skeleton, adult human
Skeleton, monkey
Skeleton, fetal human (optional) (24–7170)
Skeletons or mounted limbs (24–6484) of:
  frog
  lizard
  bird
  bat
  cat
Pencils, wax or marking
Toothpicks, serum stirring

Chemicals

Immunology and evolution kit including:
  Deep well testing plates
  Rabbit blood serum
  Human sensitizing serum
  Human serum (*Note:* not labeled as such, labeled *Test Serum III*)
  Test Serum I, II, III (human serum), IV, and V

Microscope Slides, Prepared

Chick 96 hr whole mount (Carolina E 2240)
or
(Carolina Plastomount—POM 4930 Chick Embryo Combination)
or
POM 5100 Chick Embryo Combination
Pig embryos, 8–10 mm (Carolina Plastomount—POM 7600 Pig Embryo Combination)

Laboratory 13 Mechanisms in Evolution

Hardware

Antibiotic soaked, sterile paper disks and blank, sterile paper disks
Calculator, pocket
Coins
Incubator, 37° C
Lamp, ultraviolet or ultraviolet viewbox
Pencils, wax or marking
Petri dishes, sterile
PTC (phenylthiocarbamide) taste paper
Refrigerator, 5° C
Tape

Chemicals

Nutrient agar

Living Material

Escherichia coli (E. coli) bacteria on nutrient agar petri dishes with sterile antibiotic and blank (control) disks.

Laboratory 14 Diversity I: Bacteria, Protista, and Fungi

Hardware

Microscopes, compound light
Miroscope, binocular dissecting (optional)
Microscope coverslips
Microscope slides
Microscope slides, depression (optional)
Oil immersion (optional)
Paper, lens
Pencils, wax or marking
Scalpels
Swabs, sterile
Tape, clear

Chemicals

Agar plates: sterile for student use
Methylene blue

Living Material

Kingdom Monera
  Bacteria (division Eubacteria) bacillus—rod, coccus—spheres, and spirillum (living and/or slides)
  Cyanobacteria (living and/or prepared slides)
  Gleocapsa
  Oscillatoria or *Anabaena*
Kingdom Protista
  Protozoans (living, prepared slides, video, or film loops)
Algae (living, preserved, plastomounted, prepared slides, and/or film loops)
  Green:
  Spirogyra (conjugation)
  Volvox
  Brown:
  Fucus (Carolina PB 133C) (Carolina POM 16020) or *Saragassum* (Carolina PB 138) or *Laminaria* (Carolina PB 136) (Carolina POM 16030 and POM 16025) or
  Unicellular:
  Euglena
  Diatoms
  Slimemolds: (living, video, and/or film loop)
  Dictyostelium (Carolina 15–5824) or *Physarum*
Kingdom Fungi
  Division Zygomycota
  Rhizopus bread mold (Carolina 82–1900)
  Sac fungi (division Ascomycota)
  Penicillium (Carolina 15–6155A) culture
  Club fungi (division Basidiomycota)
    Mushroom (Carolina PB 252D) (Carolina POM 16062)

Texts

Appendix C, Classification of Organisms
Picture keys for identification of pond water organisms. *A Guide to the Study of Fresh-Water Biology,* 5th ed. James G. Needham and Paul R. Needham, McGraw-Hill

Laboratory 15 Diversity II: Nonseed Plants

Hardware

Microscope, binocular dissecting
Microscope, compound light
Microscope coverslips
Microscope slides
Paper, lens

Chemicals

Water, distilled in dropping bottles

*See *Laboratory Resource Guide* for further instructions.

Microscope Slides, Prepared

Moss:
  Male (Carolina B 370A) and female (Carolina B 363) head, l.s.
  Mnium capsule, l.s. (Carolina B 365)
Fern:
  Prothallus with antheridia (Wards 91W 4860)
  Prothalliumwith archegonia (Wards 91W 4863)
  Young sporangia (Carolina 97–6329)

Models

Plastomount of moss life cycle (Carolina POM 16550)
Plastomount of fern life cycle (Carolina POM 17010)

Living Material

Fern:
  Minimarsh (Carolina 15–6630)
  Preserved fronds (Carolina 23–8200)
Moss:
  Minimarsh (Carolina 15–6630)
Primitive vascular plants:
  Equisetum with attached rhizomes (Carolina 23–8580)
  Lycopodium (Carolina 23–8480)
  Psilotum (Carolina 23–8250)

Laboratory 16 Diversity III: Seed Plants

Hardware

Forceps
Microscopes, binocular dissecting or hand lenses
Microscopes, compound light
Microscope coverslips
Microscope slides
Paper, lens
Razor blades
Scissors

Chemicals

Water, distilled in dropping bottles

Microscope Slides, Prepared

Germinated pollen; pollen grains with pollen tubes (Carolina B690A, B690AA, and B691)
Pine: male cones, longitudinal section (Carolina B495)
  female cones, longitudinal section (Carolina B499)
Series of *Capsella* embryo development (optional) (Carolina B716 to B719)

Models

Flower

Fresh Material

Fruits:
  acorns
  apples
  barley
  beans, string
  corn kernels
  grapes
  milkweed
  oats
  peaches
  peanuts, unshelled
  pear
  pineapple
  poppy capsule
  raspberries
  sunflower head
  tomato
Seeds, presoaked:
  bean seeds
  corn kernels
Pine:
  male cones
  female cones
  seeds

Living Material

Seedlings of:
  beans
  corn
Specimens of:
  Ephedra
  Ginkgo
  Zamia or *Cycas*

Laboratory 17 Diversity IV: Primitive Invertebrates

Hardware

Microscope, binocular dissecting or hand lenses
Microscope, compound light
Microscope coverslips
Microscope slides
Pans, dissecting
Paper, lens
Pieces of meat or boiled egg yolk
Scalpels
Scissors (fine)
Syracuse dishes or watch glasses
Vinegar

Chemicals

Methyl cellulose (protoslo) (see Common Materials section of Carolina Biological Supply Catalog)
Vinegar

Microscope Slides, Prepared

Ascaris lumbricoides, c.s. (Carolina Z1035, Z1037)
Grantia calcareous spicules (Carolina Z526)
Grantia, l.s. (Carolina Z510)
Hydra, c.s. (Carolina Z645) l.s. (Carolina Z665)
Obelia colony (Carolina Z690) medusa (Carolina Z691)
Planaria, w.m. (Carolina Z905) c.s. (Carolina Z920, Z923)

Living Material

Hydra (Carolina L55)
Planaria (Carolina L210)
Rotifers *Monostyla* (Carolina L301) and *Philodina* (Carolina L302)
Vinegar eels (*Turbatrix aceti*) (Ward 87 W2950)

Fresh Material

Meat, liver or boiled egg yolk; a small amount to feed *Planaria*

Preserved Material

Ascaris lumbricoides earthworm (Carolina P265C)
Hydra with:
  buds, w.m. (Carolina P61)
  spermaries (Carolina P63)
  ovaries (Carolina P64)
Representative cnidarians (Anemone—Coral Collection; Carolina P195C) (Hydrozoan Collection; Carolina P185) (Jellyfish Types; Carolina P124C)
  jellyfish (Carolina P119C to 128F)
  sea anemone (Carolina P145C to 166A)
  corals (Carolina P173 to P180)
Sponge (Carolina P35 to P55)

Laboratory 18 Diversity V: Protostomes

Hardware

Finger bowls, large (optional)
Card, index
Microscope, binocular dissecting or hand lenses
Microscope, compound light
Ink, India (optional)
Pans, dissecting
Paper, lens
Pins, dissecting
Scalpel

*See *Laboratory Resource Guide* for further instructions.

Microscope Slides, Prepared

Earthworm, c.s. (Carolina Z1251–1253, Z1255)

Preserved Material

Biomounts:
- Annelid collection (Carolina 26–1675)
- Arthropod collection (Carolina 26–2101)
- Complete and incomplete metamorphosis (Carolina 26–2374)
- Mollusk (Carolina 26–1760 or 26–1770)

Also Available as Living:
- Clam: *Glochidia* (Carolina P504), *Ensis* (Carolina P505), *Mya* (Carolina P506C)
- Crayfish (Carolina P590–595)
- Earthworm (Carolina P400)
- Grasshopper (Carolina P727)

Laboratory 19 Diversity VI: Deuterostomes

Hardware

Forceps
Pans, dissecting
Pins, dissecting
Scalpels
Scissors

Microscope Slides, Prepared

Lancelet (*Amphioxus*), c.s. (Carolina Z2735)

Preserved Material

Frog, life cycle (xenopus) (biomount) (Carolina 26–3622)
Frog (Carolina P1490–1502)
Lancelet (*Amphioxus*) (Carolina P1253C)
Sea Star (Carolina P331C to P335F)
Representative specimens:
Amphibians, frog collection (Carolina P1489C)
Bird (Carolina P1675 to 1755)
Echinoderm collection (Carolina P385)
Fish collection (Carolina P1421C)
Mammals (Carolina P1800 to 3182C)
Reptiles (Carolina P1629D to 1660C)

Laboratory 20 Structure and Organization of Plants

Microscope Slides, Prepared

C_3 leaf, c.s., maple (Carolina 97–8665), *Liriodendron* (Carolina 97–8664)
C_4 leaf, c.s., corn (Carolina 97–8760)
Monocot stem, c.s., corn (Carolina 97–8420)
Dicot herbaceous stem, c.s.
- *Ranunculus* (Carolina 97–8310) or
- Alfalfa (Carolina 97–8220) or

Dicot woody stem, c.s.
- *Liriodendron* (Carolina 97–8275) or
- *Magnolia* (Carolina 97–8278) or

Dicot root, c.s.
- *Ranunculus* (Carolina 97–7994) or

Root hairs, *Hordeum* barley, w.m. (Wards 91 W7141)

Models or Charts

Leaf, c.s. (Carolina 56–8801 to 56–8812)
Dicot woody stem (Carolina 56–8755 to 56–8760)
Monocot stem (Carolina 56–8770)
Root tip (Carolina 65–8740)

Living Material

Bean seedlings (Carolina 15–8300 to 15–8430); plant 2 to 3 weeks ahead of time
Winter twigs; collect in winter and save for future use

Preserved or Living Material

Modified leaves
Modified stems
Root systems

Laboratory 21 Water Absorption and Transport in Plants

Hardware

Beaker, large
Cork borer
Forceps
Glass tubing in 30 cm lengths
Microscopes, binocular dissecting
Microscopes, compound light
Microscope coverslips
Microscope slides
Needle, dissecting
Paper, filter
Paper, lens
Paper towels
Pasteur pipettes or droppers or long plastic pipettes
Pencil, wax marking
Petri dishes
Razor blades
Ring stand clamps; one/transpirometer
Ring stands; one/transpirometer
Rubber bands, string, or bag ties
Rubber tubing in 4 cm lengths; one/transpirometer
Rulers
Scalpels
Scissors (medium)
Spatula, micro
Test tube racks
Test tubes
Tubs or large pans

Microscope Slides, Prepared

Dicot root, cs.: Buttercup (*Ranunculus*) root, c.s. (Carolina 97–7994)
Dicot leaf, c.s.: *Oleander* (Carolina 97–8670) or Carnation (Carolina 97–8630) or Rubber tree (Carolina 97–8710)

Chemicals

Colors, food; use to dye sucrose solutions
Methylene blue, 5%; for celery stalks
Neutral red, 0.1%, in dropping bottles for root hairs
Sodium chloride (salt, NaCl), 5%
Sucrose solutions:
- 0.1 M, blue for demonstration
- 0.15M
- 0.2M
- 0.2M, green for demonstration
- 0.25M
- 0.3M
- 0.35M
- 0.4M
- 0.4M, red for demonstration

Water, distilled, in dropping bottles; for stomata, one/group

Living Material

Celery stalks
Geranium plants
Plants of choice where epidermis strips easily
Potatoes
Radish, mustard, or redtop seedlings; sprouted in petri dishes

Laboratory 22 Control of Plant Growth and Development

Hardware

Aluminum foil
Boxes, light tight cardboard: one with 7 mm square opening in center of one side, one without opening
Jars, gallon or small aquarium
Lamp, flood, 150 watt; for phototropism
Needle (sewing kind); 5 or 6 for sewing on seedlings
Paper, filter, to fit petri dishes; for germination of seeds
Pencil, wax marking
Petri dishes (covered) with aluminum foil (germination in the dark)

*See *Laboratory Resource Guide* for further instructions.

Rulers
Scissors
Tape
Thread; for sewing on seedlings

Chemicals

Acid, gibberellic, 10^{-6}M (Carolina 19–8630)
Agar, starch (Carolina 78–7320) in petri dishes with oat, wheat, or other grains
Bleach, 10% chlorine solution to disinfect seed

Living Material

Germinated seeds—radish, mustard, cress, and lettuce in petri dishes
Seedlings—mustard or bean (grown in dark and light)
Seedlings—oat, radish, or wheat
Seedlings—red top (1–2 cm tall)

Laboratory 23 Basic Mammalian Anatomy

Hardware

Bags, large plastic to fit pig
Dissecting needles
Dissecting trays
Forceps
Labels
Pencil, wax marking
Scissors, heavy
Scissors, medium
String, heavy

Models

Human torso model (Carolina 56–6400 to 56–6636)

Preserved Material

Fetal pigs (Carolina P1895 to P1905)

Laboratory 24 Comparative Vertebrate Anatomy

Hardware

Forceps
Microscope, compound light
Needle, dissecting
Pans, dissecting
Paper, lens
Probe, blunt
Scalpel
Scissors, medium

Microscope Slides, Prepared

Frog blood (Carolina H1060)
Human blood (Carolina H1155)
Bird blood (optional) (Carolina H1100)

Models

Frog (Carolina 56–4895 to 4929)
Human heart (Carolina 56–6802)
Hearts of amphibian, bird, and fish optional, vertebrate set (Carolina 56–4655)
Perch (Carolina 56–4750 or 56–4755)

Living Material

Goldfish (Carolina L1350, L1360, L1363)
Frog (Carolina L1505)

Preserved Material

Frog (Carolina P1475C to 1502F)
Perch (Carolina P1410AC to 1411F)
Pig, fetal (Carolina 1895 to 1905)
Pigeon (Carolina P1700C)

Laboratory 25 Chemical Aspects of Digestion

Hardware

Beakers, 1,000 ml; a boiling water bath
Corks, or rubber stopper; to fit test tubes
Hot plates
Incubator at 37° C or heater blocks (see Common Materials section of Carolina Biological Supply Catalog)
Pencil, wax marking
Rulers, plastic
Test tubes
Test tube holders
Test tube racks
Thermometers
Tongs, beaker

Chemicals

Albumin solution, 1%; 4 test tubes/group filled to the 2 cm mark × total no. of groups
Alpha-amylase, 1%–2% (Carolina 20–2350); 5 test tube/group filled to the 1 cm mark × total no. of groups
Benedict's reagent
Bile solution, 2.0% (Carolina 79–5000)
Biuret reagent (see Common Materials section in Carolina Biological Supply catalog)
Hydrochloric acid (HCl), 0.2%
Iodine solution
Sodium hydroxide (NaOH), 0.2%
Oil, vegetable; fat digestion
Pancreatin solution, 1%–2% (Carolina 87–8928); fat digestion
Pepsin solution, 1%–2% (Carolina 87–9378); protein digestion
Phenol red solution or 5 dropping bottles
Starch suspension—fresh
Water, distilled

Laboratory 26 Homeostasis

Hardware

Beaker, boiling water bath, Benedict's reagent
Dipsticks, urine (Carolina 69–5960 to 69–5969)
Hot plates, Benedict's test
Microscopes, compound light
Paper, lens
Pencil, wax marking
Ruler, plastic
Test tubes
Test tube holder
Thermometer to check water temperature
Tongs, beaker
Towels, paper
Water bath, boiling

Microscope Slides, Prepared

Lung tissue (Carolina 99–7745)

Models

Kidney, human (Carolina 56–6919A–6923)

Chemicals

Benedict's reagent, sugar test (see Common Materials section in Carolina Biological Supply catalog)
Glucose solutions (as serum samples)

Prepared Material

Prepared urine samples

Laboratory 27 The Nervous System

Hardware

Graduates, 10 ml
Meter stick
Microscopes, compound light

*See *Laboratory Resource Guide* for further instructions.

Microscopes, binocular dissecting
Needles, dissecting
Pan, dissecting
Paper, lens
Scalpel
Scissors, medium

Microscope Slides, Prepared

Spinal cord, c.s. (Carolina 98–6740; H1535; H1537)
Nerve, c.s. (Carolina H1578)

Models

Vertebrate brains, comparative set (Carolina 56–4644 or 56–4650)

Living Material

Live frog (Carolina L1493W–1502)

Preserved Material

Pig, fetal (Carolina P1895 to P1905)
Sheep brain (Carolina P2100S)

Laboratory 28 Senses

Hardware

Beakers, 1,000 ml
Block, small styrofoam
Bottle, wash; wash flies in taste test
Cards, afterimage (22 × 28 × 8 cm in center)
Card, index
Cups, small, paper or microbeaker, plastic
Fork, tuning or 2 spoons; hearing test
Hot plate or hot tap water
Penlight; pupillary reflex
Plates, deep well testing; blowfly taste
Pins or needle (sharp); pain; clean
Ruler, metric; near point
Scissors or hair pins or large paper clips; touch
Sticks, applicator; with Vaseline and flies; fly taste test
Stool, rotating; balance
Thermometer; to check temperature for heat and cold
Wiregrid, 2 cm squares, 1/2 cm grid; pain test

Models

Ear (Carolina 56–6966 or 56–6967)
Eye (Carolina 56–6941 to 6956)
Skin (Carolina 56–7664 to 7678)

Chemicals

Sugar concentrations
Each student should obtain his or her own fresh solutions and place them in fresh cups. *Caution*—do not share container or liquids.
Galactose solutions: 5 ml of each concentration × total no. of students
  0.05M
  0.1M
  0.2M
  0.4M
  0.8M
Glucose
  0.05M
  0.1M
  0.2M
  0.4M
  0.8M
Sucrose
  0.05M
  0.1M
  0.2M
  0.4M
  0.8M

Living Material

Blowflies attached to sticks with Vaseline

Preserved Material

Grasshopper (Carolina P727)
Squid (Carolina P526 to 530F)
Fish (optional) (Carolina P1410AC to 1411F)

Demonstration Material

Arthropod (insect) eye under binocular dissecting microscope

Laboratory 29 Musculoskeletal System

Hardware

Forceps
Microscope, compound light
Microscope coverslips
Microscope slide
Needles, dissecting
Pans, dissecting
Paper, lens
Rulers, millimeter
Scalpel
Scissors

Microscope Slides, Prepared

Compact bone, c.s. (Carolina H780 and 782)
Hyaline cartilage (Carolina H680)
Skeletal muscle, l.s. (Carolina H1310)

Models

Skeletons of:
  Bird (Carolina 24–510 to 5220)
  Cat (Carolina 24–5850 to 24–5970)
  Frog (Carolina 24–3710 to 3840)
  Human (Carolina 24–6725 to 7001)
Chicken bones—untreated, decalcified, deproteinized

Chemicals

Acetic acid, 5%–10% (Carolina 84–1313)
ATP muscle kit
Glycerol
Ion solution (frog ringers)

Living Material

Earthworms (Carolina L–400)
Frogs (Carolina L1496 to 1502)

Preserved Material

Frogs (Carolina P1491C to 1502F)
Glycerated muscle (Carolina 20–3520)

Laboratory 30 Animal Development

Hardware

Bowls, 5-in finger, eggs (can use cut egg cartons or paper towels)
Forceps with fine points
Needle, dissecting
Microscopes, binocular dissecting
Petri dishes or Syracuse dishes
Scissors, fine points

Microscope Slides, Prepared

Lancelet (amphioxus) cleavage (Carolina ZL 1–04)
  Morula (Carolina ZL 1–05)
  Blastula (Carolina ZL 1–07)
  Gastrula (Carolina ZL 1–08)

Models

Human development (Carolina 56–3115 to 3128) early embryonic, 4 week, 8 week

Chemicals

Chick ringers

*See *Laboratory Resource Guide* for further instructions.

Preserved Material

Frog eggs
  Fertilized (Carolina P1530AF)
  Cleavage (Carolina P1530BF–FF)
  Blastula (Carolina P1530GF–HF)
  Gastrula (Carolina P1530KF, MF, NF)
  Neurula (Carolina P1530PF, QF, RF)
  Tail Bud (Carolina P1530SF)

Living Material

Live chick embryos 38 and 72 hour (purchase locally)
Raw chick eggs

Laboratory 31 Animal Behavior

Hardware

Aluminum foil
Aquarium; 15 gal; for "Instinctive Behavior in Fish (Optional)"
Bottles, squeeze for distilled water
Cages; 4 bird
Droppers, medicine
Jars, two 4 liter glass; for "Instinctive Behavior in Fish (Optional)"
Lamps, phototaxis
Lenses, magnifying
Needles, dissecting
Nesting bowls
Nesting materials
Paper, white in sheets, phototaxis background
Pencils, wax marking
Petri dishes or Syracuse dishes; flatworm taxis
Rods, for fly attachment
Stoppers, cork or screw cap test tubes
Stopwatches; 3
Test tubes with screw caps or stopper; flatworm behavior
Test tube racks
Tubing, clear plastic or glass (convenient lengths) for fly behavior
Tubes, Y shape; fly behavior

Chemicals

Water, distilled in squeeze bottles

Living Material

Fish, 2 species (optional); purchase locally from fish store
Flies attached to rods
Fruit fly (wild type) culture
Planarians (Carolina L210–221)
Ring doves; purchase at pet store 3–4 weeks prior to lab

Fresh Material

Banana, mashed ripe (or a substitute food)
Liver, raw cut in small pieces or use beef

Laboratory 32 Symbiotic Relationships

Hardware

Dishes, Syracuse or watch glasses
Droppers, medicine
Microscopes, compound light
Microscope coverslips
Microscope slides
Needles, dissecting
Pans, dissecting
Scalpel or razor blades
Scissors
Swabs, alcohol
Thread, cotton
Toothpicks
Water, distilled

Microscope Slides, Prepared

Lichen thallus (Carolina B293 or 97–5200T)
Necator americanus (Carolina PS2392 and 2394)
Plasmodium (Carolina PS600 and 620)
Root nodules with bacteria (Triarch, Inc. K3–94b)
Schistoisoma mansoni (in Copula) (Carolina PS1318)
Taenia pisiformis (Carolina PS1825 and 1828)
Trichinella (Carolina PS2420, 2430, 2435)
Trichonympha and *Pyrsonympha* (Carolina 98–3021) (substitute for live termites)

Models (Plastomounts)

Lichen types (Carolina POM 16070)

Chemicals

Sodium Chloride (NaCl), 0.9%

Living Material

Frogs (optional) (Carolina L1493W to 1502A)
Lichens (Carolina 15–6400 to 6445)
Rhabditis (Carolina L268)
Termites (Carolina L740 to 741)

Preserved Material

Lichen types (Carolina PB285 to 291)
Liver fluke (Carolina P225C sheep, P228 human)
Meat infected with *Trichinella* (Carolina P294)
Plant with root nodules (Carolina POM 17570)
Tapeworm (Carolina P245C)

Laboratory 33 Analysis of an Ecosystem

Hardware (General)

Jars, collecting; various sizes
Meter sticks
Microscope, binocular dissecting and/or hand lenses
Microscope, compound light
Microscope coverslips
Microscope slides
Thermometers, air and water
Pails and/or tubs
pH paper or pH meters

Chemicals (General)

Alcohol, isopropyl, 80%, or ethyl alcohol, 70%

Text

Classification keys for invertebrates, birds, mammals, and plants

Hardware (Terrestrial Ecosystem Analysis)

Funnel, Berlese
Equipment for quadrat construction
  String; 1 m pieces
  Stakes, corner
Nets, insect; aerial and sweeping as available
Petri dishes and tubes with nutrient agar (optional)
Shovels and/or trowels
Thermometers, soil

Hardware (Aquatic Ecosystem Analysis)

Disk, Secchi
Dredge, Ekman
Nets, aquatic
Net, Wisconsin plankton
Trap, Juday plankton
Sampler, Serber Stream
Sampler, Van Doren
Scraper, Needham
Kits, water test for chemical analysis
Waders, hip

*See *Laboratory Resource Guide* for further instructions.

Laboratory 34 Effects of Pollution

Hardware

Beakers; 500 ml, 100 ml, 50 ml
Cuvette for spectrophotometer
Droppers, medicine; pesticide and thermal pollution
Hot plate or other heat source; warm water; thermal pollution
Microscopes, binocular dissecting
Microscopes, compound light
Microscope slides
Microscope coverslips
Paper, pH; 3 to 5 strips per group
Pencil, wax marking
Dishes, Petri or Syracuse
Petroleum jelly
Spectrophotometer
Test tubes, spectrophotometer (cuvettes)
Test tube holder
Thermometers
Tongs, beaker; to remove hot water from the hot plates
Toothpicks or applicator sticks for Vaseline

*See *Laboratory Resource Guide* for further instructions.

Chemicals

Carmine solution (aqueous in dropper bottles), 0.1%
Sulfuric acid (H_2SO_4), 5% solution
Water, normal spring or other nonchlorinated water; large volumes for each culture
Water, low and high pesticide concentrations
Water, for *Daphnia,* pH 4 and pH 2

Living Material

Algal culture:
  Daphnia magna
  Gammarus
Hay infusion cultures:
  control
  overenrichment
  pesticide (low and high concentrations)
  acid pH (low and high concentrations)
Petri dishes containing seeds watered with:
  spring water (control)
  low and high pesticide concentrations
  low and high acid concentrations

Name _____ *Section* _____ *Date* _____

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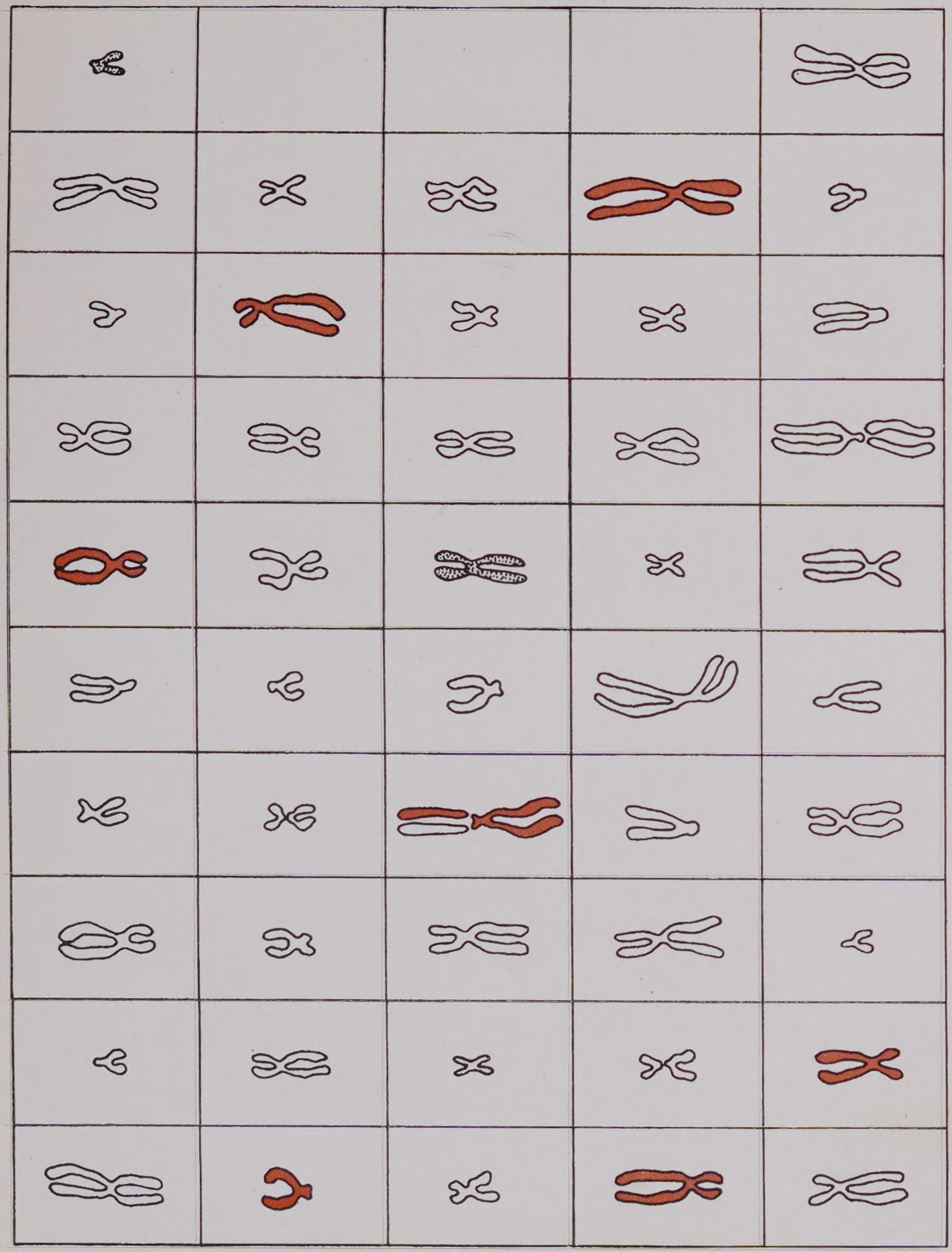

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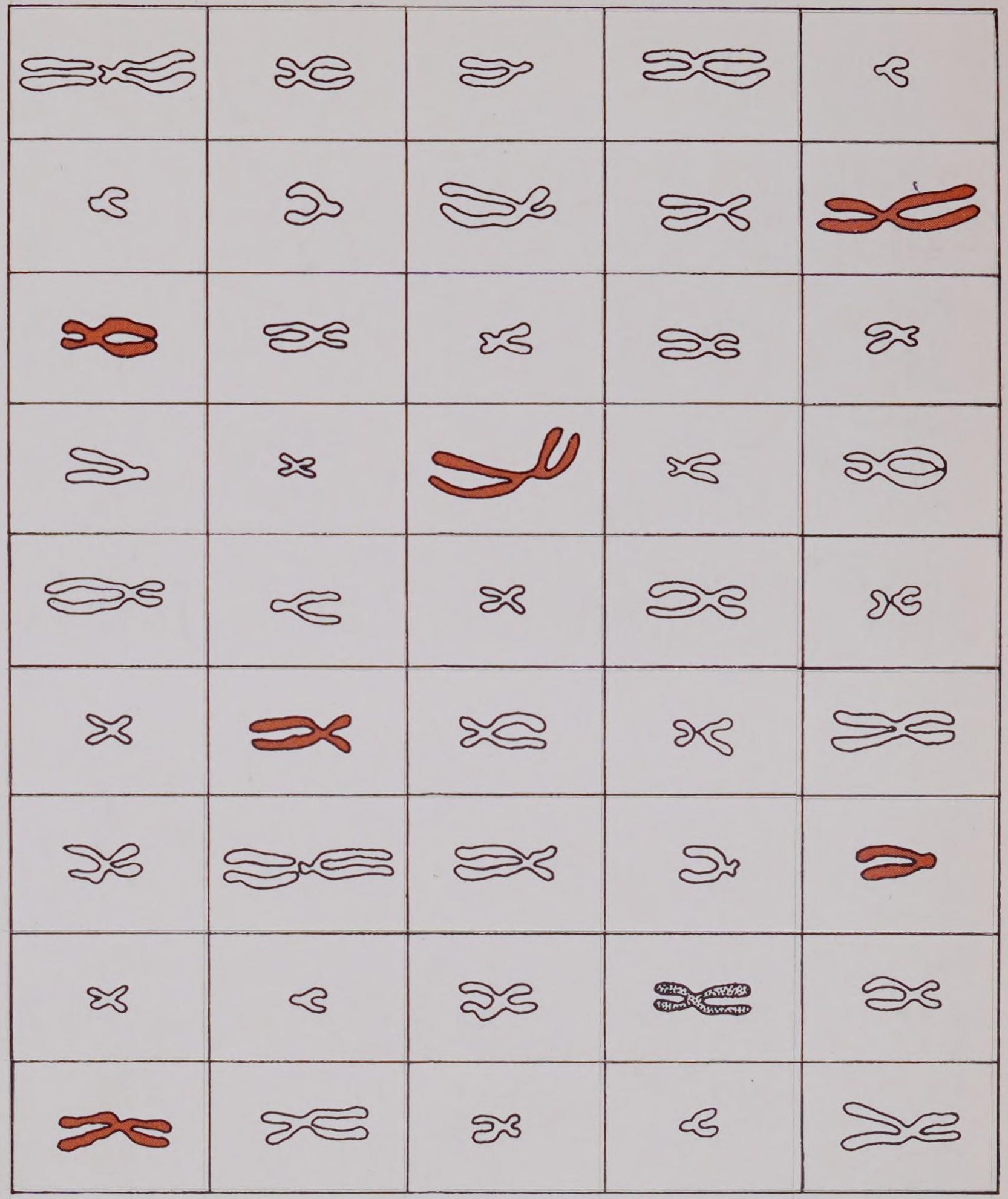

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